BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

HEREDITY AND ORGANIC SYMMETRY IN ARMADILLO QUADRUPLETS.

I. Modes of Inheritance of Band Anomalies.

H. H. NEWMAN.

Introduction.

Since the winter of 1909, when I first secured and began the study of advanced polyembryonic fetuses of the nine-banded armadillo, I have been struck not only by the striking resemblances among the individuals of a set of quadruplets but also by certain equally striking differences. It was early noted that well defined anomalous arrangements of scutes in the armor bands were sometimes repeated with closely similar detail in two or more fetuses, and were totally absent in others. Occasionally all four fetuses of a set showed a highly localized anomaly, but differed materially in the extent of the irregularity and in its symmetrical relations.

The full significance of these conditions did not dawn upon me, however, until some years later, when a set of quadruplets was obtained in which the band anomaly in question was found to be present not only in all of the four fetuses but in the mother as well. Previous to this time only a few anomalous sets had been studied and these happened to have normal mothers, a circumstance which led to the belief that these characters were not strictly inherited from parents but were merely predetermined early in embryonic life before the separation of the four embryos from the originally single embryonic vesicle. The finding of one unequivocal case of the direct inheritance from the mother of the anomalous character stimulated a new interest in the problem and made it necessary to collect a large amount of new

data. Accordingly in the winter of 1911 I journeyed from Chicago to Texas and spent about a month in the armadillo country collecting material, obtaining nearly two hundred advanced polyembryonic sets together with the armor of the mothers.

A study of this material by my students and myself has brought to light a situation so interesting, but withal so intricately complex, that I almost despair of being able to render an intelligible account of it. Yet I cannot but be impressed with the uniqueness of the data and with the fact that it contains clews, afforded by no other material, as to methods of attacking some of the problems of the mechanics of hereditary transmission and of the development of organic symmetry.

In presenting the results of the study of the heredity of band and scute anomalies I have found that the problems of heredity, and that of the symmetrical distribution of these inherited characters among the quadruplet embryos, are inseparably parts of a larger problem that has to do with the mechanics of organic symmetry. When, for example, it is found that a bilateral anomaly in a mother is inherited unilaterally in the various offspring and appears in reversed symmetrical relations in twins, we have something more than a case of simple inheritance. When again we find a unilateral anomaly in one pair appearing bilaterally without reversed symmetry in the opposite pair we see the problem in a more complex form. When, still further, we note that an anomaly of a single scute in the mother is inherited as such in some of the offspring and as a whole series of anomalous scutes (a band anomaly) in others, we begin to suspect that the problem is too intricate for any simple solution and that only in certain of its aspects is it likely to yield to analysis. Yet it is our duty to carry the analysis as far as our data allows.

That the circumstance of polyembryony vastly complicates the already sufficiently complex problems of heredity can scarcely be denied, but it is confidently hoped that the new relations introduced by this unique type of development may throw light from a different angle upon certain phases of heredity that are now quite obscure.

Before the reader can appreciate the significance of the data on heredity and organic symmetry, it will be necessary for him to be made familiar with the character of the material and its specific frequency and distribution.

Material and Methods of Study and Presentation.

The material for this investigation consists of nearly two hundred sets of quadruplet fetuses in advanced stages, showing the definitive arrangement and number of scutes in the armor. In every case the shell of the mother was obtained and preserved so that there is on hand complete data on uniparental inheritance of armor characters. The fetuses and the respective mothers make up a collection of nearly one thousand individuals, which is unquestionably a representative sample of the species.

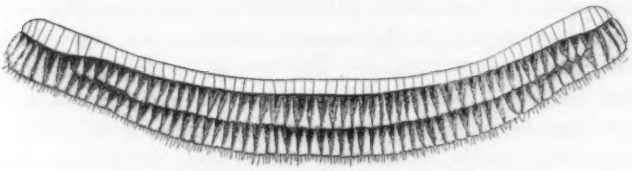
Counts of scutes and records of anomalies have been made independently by at least two workers and all differences or discrepancies have thus been checked and corrected, so that the probability of error and personal bias have been eliminated.

Since the anomalies here dealt with are rare, occurring in only about three per cent. of individuals, I have made an examination of the preserved armor of 1,800 adults that formed the stock of a single dealer. This was done in order to determine the limits of diversity and the specific distribution of the anomalies. A complete record of all these anomalies has been made in the form of a pictorial diagram in Table *A*, 1, 2 and 3.

In order that the reader may understand the nature of the anomalies it will be necessary to give a brief statement of the normal relations of armor units. The primary unit of the armor is a threefold complex consisting of a bony plate, covered by a primary epidermal scale of scute, and a group of hairs that are imbedded in the bony plate and perforate the scute. For convenience this complex is called a "scute." In young fetuses the outline of the epidermal scute is all that can be seen; but this visible unit stands for the whole complex. These scutes or armor units of the carapace are arranged differently in the three main subdivisions of the armor, the pectoral, banded and pelvic shields. In the pectoral and pelvic regions the bony plates are arranged like tiles and form a rigid immovable shield. Naturally

the primary scutes are arranged in more or less regular transverse rows, but these rows seldom run straight across the shield. Instead it is commoner for a row that starts single on the margin to become double for some distance where the bulge of the shell is greatest, then to be single again toward the middle. Again a row may start double laterally and be single for a short distance toward the middle. Irregularities in scute rows are commonest near the posterior border of the pectoral shield next to the banded region.

In the banded region the arrangement of scutes is quite different. The bony plates are elongated antero-posteriorly to form rows of units, in general appearance somewhat like the keyboard of a piano. The posterior margin of the first band is free from and slips over the anterior margin of the second, an arrangement that lends flexibility to the armor. The second band bears a similar relation to the third and so throughout the nine movable bands. Each bony plate is accompanied by and partially covered by an elongated, wedge-shaped primary scute. Figs. 1 and 3 represent two bands removed whole from the armor. The typical band, however, is composed of an even array of scutes arranged in a single row from margin to margin. Only one third of one per cent. of the 16,200 bands examined are in any way irregular in the arrangement of the armor units. The rare exceptions, however, are of especial interest and constitute the anomalies that are the subject of the present study. These peculiar bands belong for the most part to the types, the details of which are shown in Figs. 1, 3 and 5. They consist of bands that are partly single and partly double. In Fig. 1 is seen a very common type of bilateral anomaly in which a few scutes on each margin constitute the single part of the band, while the main central region is double. In Fig. 3 we see an equally common condition in which the double part of the band is confined to a number of scutes starting at some distance from the margins but stopping short of the middle portions of the band. Similar conditions of greater or less extent are found unilaterally as frequently as bilaterally. Now these irregular bands are in no sense abnormalities or products of injury, but are of the same nature exactly as are the irregulari-



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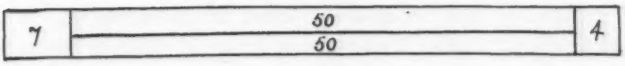

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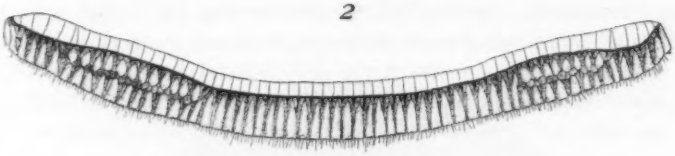

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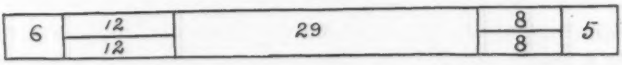

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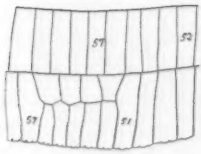

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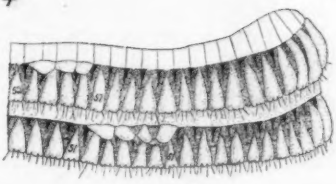

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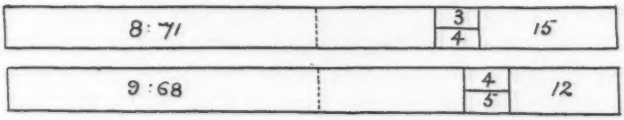


7

ties of scute rows in the adjacent pectoral shield, where regularity is rare and irregularity the typical condition. In about three per cent. of individuals the irregular conditions typical for the posterior part of the pectoral shield invade the adjacent parts of the banded region. That this is the correct interpretation of the anomalies in the banded region is evidenced by the fact that out of 84 band anomalies recorded for this study 73 occur in the first band, which is contiguous to the pectoral shield. Of the remaining eleven cases 7 occur in band 2, 1 in band 3 and 1 in band 8, which is near the other irregular region, viz., the pelvic shield. Irregularities never occur in the middle bands of the carapace, which are farthest from the irregular pectoral and pelvic shields. Another way of interpreting the facts is to look upon the arrangement of scutes in the banded region as due to mechanical adjustments of the primordia of armor elements to flexures during embryonic development. Typically the banding confines itself to the abdominal parts of the armor but occasionally comes in a little farther forward or a little farther back and thus includes parts of the pectoral and pelvic carapace with the typical peculiarities of scute arrangements of these regions. As a result we have these anomalous cases in which the first or second band has the characteristics, doubling, etc., of the pectoral region, and the posterior bands have similarly the characteristics of the pelvic region.

An irregularity in a given band of the banded region may involve as few as two or even one scute. Such an irregularity may consist of a single scute or two in an otherwise double band or a double scute or two in a band otherwise single. The latter situation is much the commonest of all anomalies studied. Sometimes the anomaly manifests itself by a more or less complete longitudinal or diagonal splitting of a single acute. Such conditions are to be dealt with separately as there is a considerable mass of interesting data on the inheritance and distribution of these anomalous double scutes. It is impossible, however, to deal with the inheritance of anomalous band conditions without discovering the intimate genetic connection that exists between band and scute anomalies, for sometimes a scute anomaly in a parent reappears as a band anomaly in offspring and vice versa.

After many experiments in tabulating the occurrence of band and scute anomalies I have adopted a simple pictorial scheme which will readily explain itself on examination of the diagrammatic Figs. 2, 4 and 7 which are simplified representations of the scute conditions shown in Figs. 1, 3 and 5 respectively. The numbers of scutes in both double and single regions are represented by arabic numerals and the number of the band is indicated sometimes, as in Table *A*, 1, 2 and 3, by the abbreviation Bd. 1 or Bd. 2 just above the margin of each band, and sometimes as in table B by a single arabic numeral followed by a colon and the total number of scutes in the band. When the quadruplet fetuses and the mother are dealt with, the number of the set is indicated as A.101 ♀ or K.40♂, indicating at the same time the sex of the litter; the bands of the mother are labeled M and

those of the fetuses I., II., III. and IV. When the mother or any fetus is not tabulated the inference is that no anomaly is present in the omitted individuals. Other schemes of tabulation, such as the circular figures and those showing double scutes will be explained in the proper place.

SPECIFIC DISTRIBUTION AND FREQUENCY OF BAND ANOMALIES.

Little need be added to the data given in Table *A*, 1, 2, 3, which shows the anomalies found in 1,800 adult specimens. On previous occasions I have made records of over 1,000 other specimens and have failed to find any other types of diversity than those shown in this table. So it may be considered as established that we have before us in this collection an adequate representation of the diversity of band anomalies and their distribution among the various bands. Although no two irregular bands in unrelated individuals are just alike there are certain well-defined classes of anomaly such as the bilateral (symmetrical or asymmetrical) and the unilateral. There are types single on the margins and double in the middle; there are types double at the margin and single in the middle; and there are mixed types.

Specimens 1–14 show various types of anomaly in which the bands are single at the margin with bilateral regions of doubling. Specimens 15 to 35 show unilateral expressions of the same types of anomaly. Specimens 36–42 show various mixed types, which are perhaps reversals of anomalies of the two sides of the individuals. Fig. 42 is an especially interesting case of such a reversal of symmetry, for the right and left halves of the band are duplicates but are not mirror-image effects. They bear the same relation to each other as the reversed finger prints found occasionally on the right and left index fingers of human duplicate twins, as shown by Wilder.

Some of the cases of exact bilateral symmetry, such as those in specimens 5, 7 and 36 are very significant, and there are all degrees of inexact bilateral duplication of anomalies ranging from specimens 12, 4, 8, 11, 14 down to specimens 13, 10, 9, 6, etc.

It must not be forgotten that there are no two anomalies alike in nearly three thousand specimens taken at random. When, therefore, we find, as we soon shall, exact duplicate anomalies in two or more fetuses in a set we shall not be able to explain them as coincidences.

Having made clear the nature of the anomalies and their diversity and distribution in the species, we are now in a position to examine the data on the inheritance of these characters.

TABLE A1.

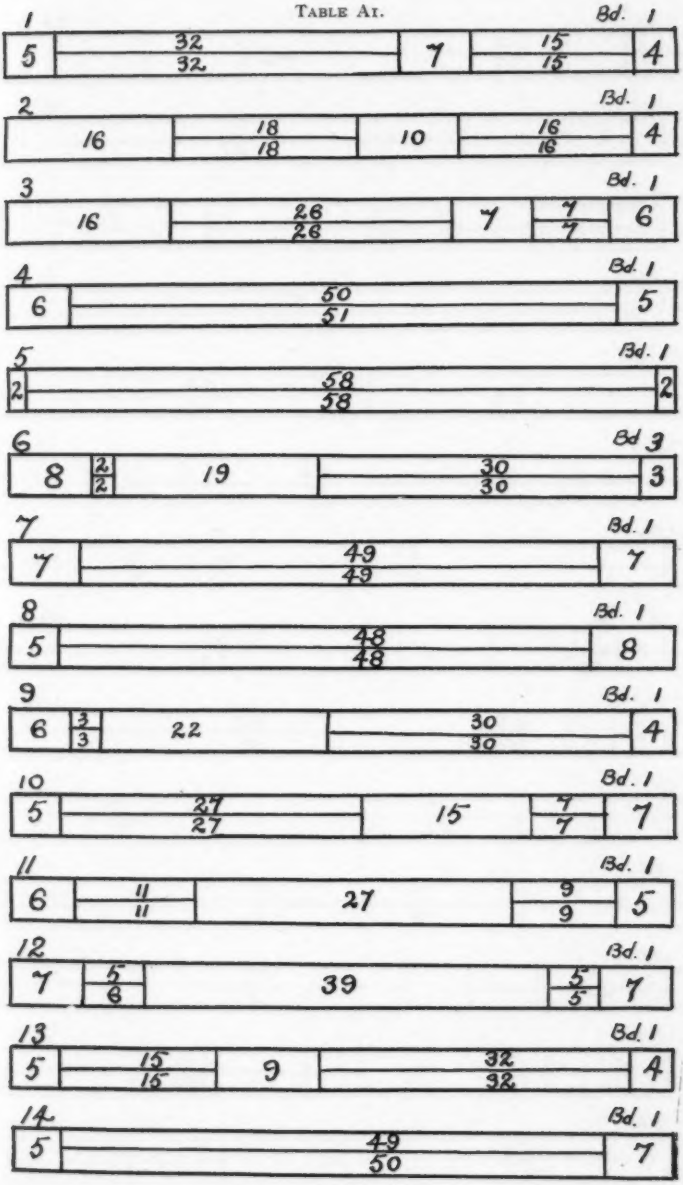
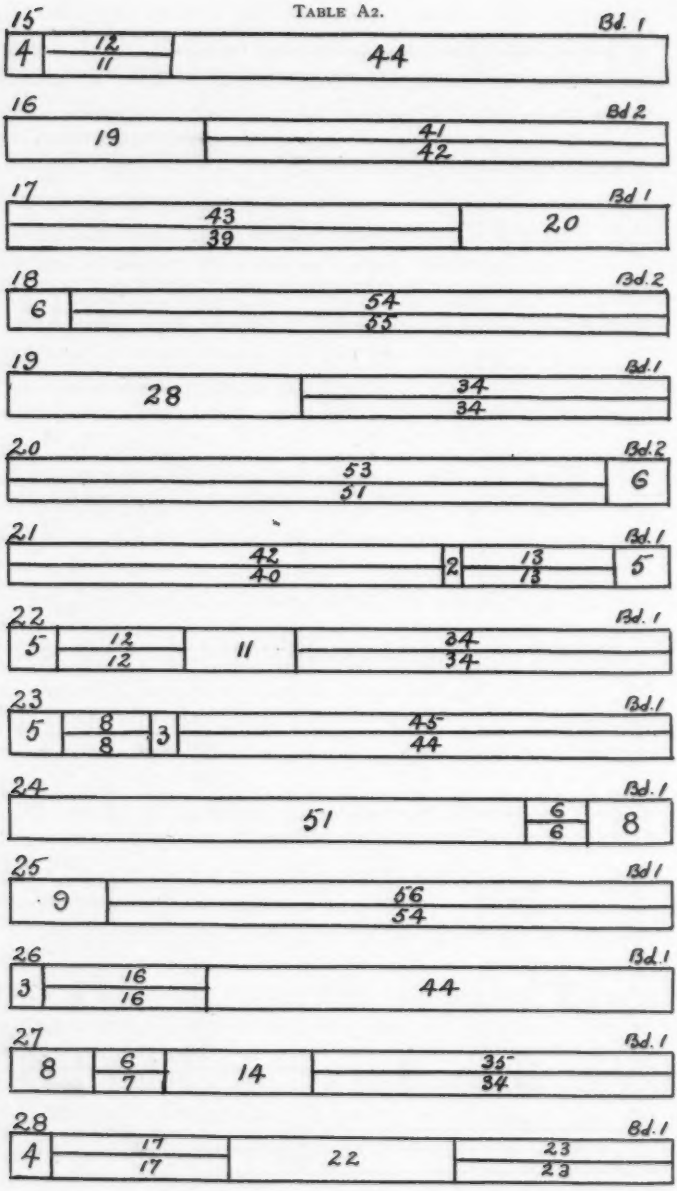

1
Bd. 1
5
32/32
7
15/15
4
2
Bd. 1
16
18/18
10
16/16
4
3
Bd. 1
16
26/26
7
7/7
6
4
Bd. 1
6
50/51
5
5
Bd. 1
2
58/58
2
6
Bd 3
8
2/2
19
30/30
3
7
Bd. 1
7
49/49
7
8
Bd. 1
5
48/48
8
9
Bd. 1
6
3/3
22
30/30
4
10
Bd. 1
5
27/27
15
7/7
7
11
Bd. 1
6
11/11
27
9/9
5
12
Bd. 1
7
5/6
39
5/5
7
13
Bd. 1
5
15/15
9
32/32
4
14
Bd. 1
5
49/50
7

TABLE A2.

15
Bd. 1
4
12
11
44
16
Bd 2
19
41
42
17
Bd 1
43
39
20
18
Bd. 2
6
54
55
19
Bd. 1
28
34
34
20
Bd. 2
53
51
6
21
Bd. 1
42
40
2
13
13
5
22
Bd. 1
5
12
12
11
34
34
23
Bd. 1
5
8
8
3
45
44
24
Bd. 1
51
6
6
8
25
Bd 1
9
56
54
26
Bd. 1
3
16
16
44
27
Bd. 1
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Bd. 1
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17
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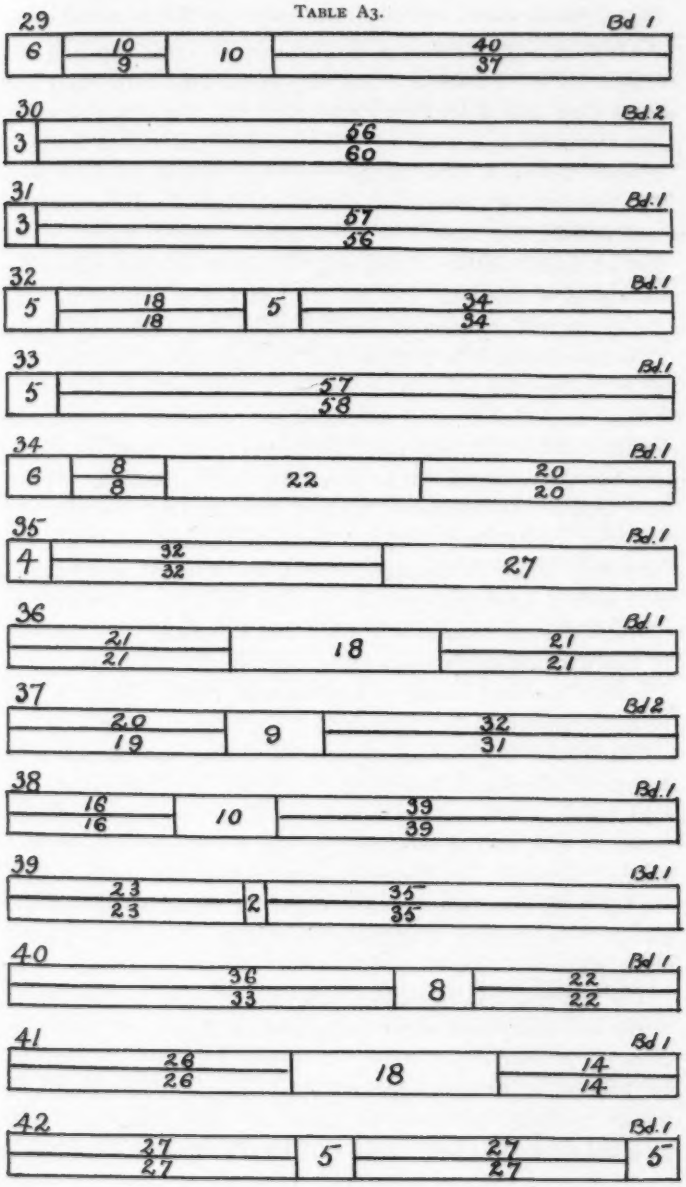
23
23

TABLE A3.


29
Bd 1
6
10/9
10
40/37
30
Bd.2
3
56/60
31
Bd.1
3
57/56
32
Bd.1
5
18/18
5
34/34
33
Bd.1
5
57/58
34
Bd.1
6
8/8
22
20/20
35
Bd.1
4
32/32
27
36
Bd.1
21/21
18
21/21
37
Bd2
20/19
9
32/31
38
Bd.1
16/16
10
39/39
39
Bd.1
23/23
2
35/35
40
Bd.1
36/33
8
22/22
41
Bd.1
26/26
18
14/14
42
Bd.1
27/27
5
27/27
5

THE INHERITANCE AND DISTRIBUTION OF BAND ANOMALIES.

After a study of over 150 of the most advanced sets of quadruplets in my collection I am convinced that both band and scute anomalies are strongly inherited. In every case in which a mother exhibits a band or a scute anomaly, a related anomaly is found in one or more of the offspring. If the character were not inherited as a dominant, one would expect some exceptions to this rule, but none have been found. When, therefore, we find an equal number of offspring of normal mothers exhibiting anomalies of the same sort we are justified in concluding that the characters represent a heritage from the unknown fathers. This assumption is farther justified by the finding that the characters in question are neither sex-limited nor sex-linked.

For our purposes, then, the data here published are adequate in that a study of uniparental inheritance reveals fully the modes of inheritance that obtain for band and scute anomalies. Whatever genetic relations are found to hold between mothers and offspring would doubtless hold for fathers and offspring. The only unfortunate complication that is encountered is in connection with a small per cent. of cases in which both fathers and mothers possess anomalies. A few sets of fetuses are obviously of this dual anomalous parentage, and we can, by knowing the maternal anomaly, make a well-founded conjecture as to the probable nature of the anomaly in the unknown father.

Whether or not I am justified in assuming that the study of maternal inheritance reveals the essential facts concerning the inheritance of the characters in question, can be settled only by breeding and, as has been pointed out in extenso in an earlier paper (Newman, '13), breeding experiments with the armadillo are at present totally impracticable. Consequently, we are forced to rely upon a study of inheritance from one parent, the mother.

The nature of the anomalies is such that I have been unable to devise any really convenient method for tabulating the facts that must be known about them. It seems necessary to consider each case of inheritance separately, and this may be done without undue prolixity because the cases are not numerous. The pictorial method appears to be well adapted for the data, but it

would be too tedious a task to draw each anomaly in detail after the fashion of Figs. 1 and 3. Instead I shall use the diagrammatic form seen in Figs. 2 and 4, which give all the necessary information. The best method I have been able to devise for showing the symmetrical or asymmetrical relations of the anomalies and their distribution among the quadruplet fetuses of a set is that shown in Figs. 9–16, in which the anomalous bands are placed as though within the embryonic vesicle. The reader must remember that the structures studied are integumentary units, that the embryonic vesicle is so inverted that the ectoderm forms the inner lining and the endoderm is on the exterior.

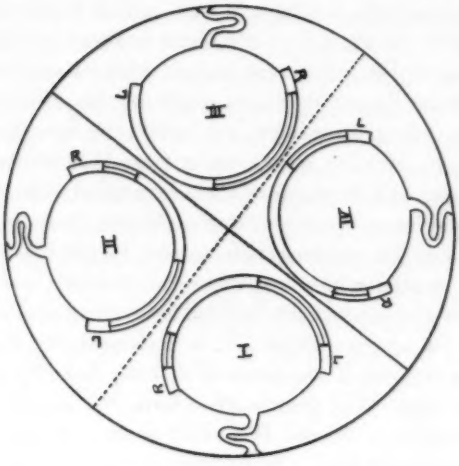
The diagram (Fig. 8) shows the relative positions of the band anomalies of the fetuses in set 87 (Fig. 9). Here we have an equatorial cross section of an advanced vesicle with the body of each fetus severed at the first armor band where the anomaly occurs. The ventral surfaces face outward and are attached by their respective umbilical cords to the placental portion of the vesicle. The dorsal aspects are turned inward. Right and left sides of each fetus are indicated. The reader must imagine himself in the center of the vesicle facing outward toward each fetus. In order to simplify this type of diagram, I have found it best to ignore all structures but the band in question and to represent the latter as though straightened out against the periphery. The reader will understand the adopted form of diagram after a comparison of Fig. 8, which represents nearly the actual relations present in set 87, and Fig. 9, which is a diagram of the same set showing only the bands that are of interest in this study. In both figures the solid quadrant line divides the twins I. and II. from twins III. and IV. and the dotted line separates twin individuals, I. from II. and III. from IV. In the actual vesicle these quadrant lines are occupied by amniotic partitions that hermetically isolate each fetus from the others. When the anomaly is found in the mother also the material condition is indicated in a straight band diagram beside or beneath the circle showing the offspring. When any individual shows an anomaly in more than one band, the more anterior anomaly is shown in the circle and the more posterior anomaly in a concentric sector outside of the circle, as in Fig. 9.

Of the twenty-six sets of fetuses one or more individuals of each of which show band anomalies, twelve came from mothers that also showed either band or acute anomalies, and fourteen came from mothers that showed no anomaly. Presumably the anomaly has been inherited from the father in these fourteen cases.

Of the twenty-six sets showing band anomalies exactly half are male and half female, which demonstrates that the characters dealt with have no sex-limitation or sex-linkage. Males and females inherit equally strongly from the mothers, for of the twelve sets of offspring in which the mother also shows an anomaly, five are of the female and seven of the male sex. In the case of four sets with anomalous mothers and similarly in four sets with normal mothers, I shall follow the plan of placing a circular diagram and the verbal description and analyses close together in the text. The remaining sets are tabulated in somewhat more compact form and placed at the end of the paper. Those in Table *B* are the eight remaining sets that have the anomaly in both mother and one or more offspring; those in Table *C* are the remaining ten sets with band anomaly in one or more fetuses but with normal mothers. Each set is placed in a separate block and labeled at the upper left-hand corner, with the number of the set and the sex of the young, as *C* 29 ♂. The number of the band showing the anomaly is indicated in two ways, as bd. 1 or bd. 2 placed next to or on the band, or by a number placed on the band followed by a colon and the total number of scutes in the band, as 7: 65, meaning band 7 having a total of 65 scutes. In bands more or less broken by doubling the number of scutes in each part is indicated. In the case of short series of double scutes or one double scute, the number placed on the element, together with the arrow, indicates the numerical position of the element from either margin, or from the middle, which is indicated by a dotted line.

Set K. 87, ♀ (Figs. 8 and 9).

This set shows more certainly than any other the direct inheritance of a material band anomaly by all of the offspring. In the mother the anomaly consists of a unilateral local doubling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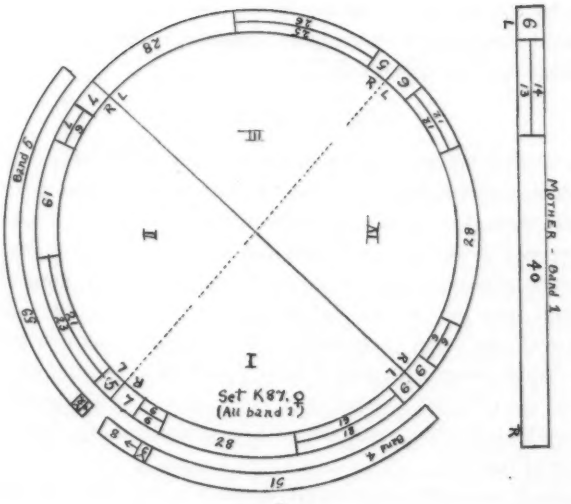
II
II
I

8


III
II
IV
I
Set K87.9
(All band 3)
Band 5
Band 4
Mother - Band 1
40

9

of band 1, confined to the left half of the band and almost identical with the left half of the band pictured in Fig. 3. The band begins on the left lateral margin with six single scutes, is then double for 14–13 scutes and single for the remainder of the series. No other anomalies are exhibited by the mother. Fetuses I., II. and IV. show similar anomalies of band 1, but they are unequally bilateral in their expression (like Fig. 3), all three showing more scutes double on the left than on the right. Fetuses I. and II. are strikingly similar in the distribution of single and double scutes and show no reversal of symmetry. Fetus IV. is the most nearly bilaterally symmetrical in the disposition of its scutes. Fetus III. is unilateral like the mother, but shows a reversal of symmetry in that the doubling is confined to the right side. The symmetry of fetus III. is also a reversal of that of fetuses I., II. and IV., which show a preponderance of doubling on the left side. In addition to the anomalies referred to, fetuses I. and II. show scute anomalies of bands 4 and 5 respectively that do not appear to be traceable to the mother and have presumably come from the father. This is one of the rare sets in which it is probable that anomalies came from both sides of the family. The double scutes of I. and II. are in a reversed symmetry to each other, being near the right in fetus I. and near the left in fetus II.

Set K. 30, ♀ (Fig. 10).

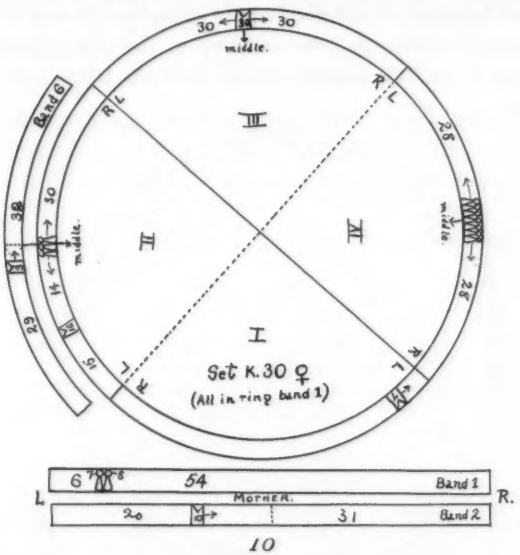
This set is of importance in that it deals with short rows of double scutes ranging from 2 to 7 elements and serves to emphasize the genetic connection between plate and scute anomalies.

The mother has in band 1 in the positions 7 and 8 from the left two adjacent double scutes forming a band anomaly of minimal size but of the same character as the short double regions of the band pictured in Fig. 5. If only one scute had been double-tiered, we would have called it a "scute" anomaly instead of a "band" anomaly. There is also in the mother an independent double scute situated 10 places to the left of the middle of band 2.

Fetus I. has a double scute in band 1 in exactly the position of the first of the two double scutes of the mother. This seems

to indicate that a double scute and an incipient double band are genetically equivalent.

Fetus II. has in the exact middle of band 1 a short series of three double scutes like the two which are near the left margin of the mother; and in the same band, midway between the middle and left band margin, one double scute. In band 6 of the same fetus there occurs at a point three scutes to the left of the middle another double scute like that in band 1. Note that all asymmetrical anomalies are on the left side as in the mother.



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Fetus III. has one double scute in the exact middle of band 1, or in a position identical with the short double band in fetus II.

Fetus IV. has a double region of 7–7 scutes in the exact middle of band 1. This doubling is simply a more pronounced case of the doubling shown in the mother as in fetuses II. and III.

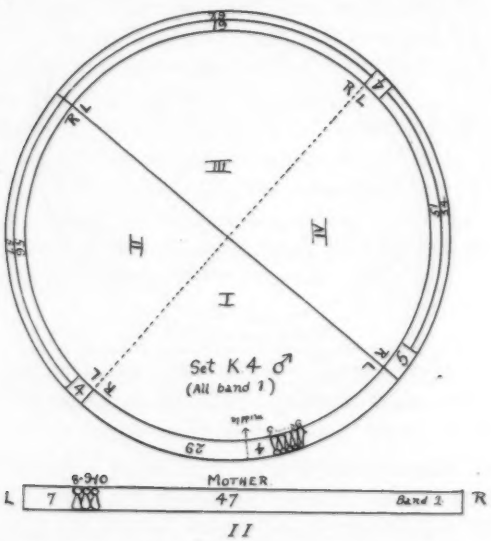
The points of interest illustrated by this set are:

1. In mother and offspring the unilateral symmetry is the same throughout except that there is a tendency for the anomaly to shift to the center of the band. This shifting of lateral anomalies to the center is a peculiar type of symmetry reversal in which the central and lateral portions of each half band become

reversed. That this is the correct interpretation of the situation is amply shown by many cases shown subsequently.

2. There occur several examples of the reduplication of a single inherited unit, sometimes involving a single band, sometimes two or more. Doubtless the anomalies of band 1 and band 2 in the mother represent a merely reversed reduplication of the same inheritance unit. Similarly in fetus II. the series of three double scutes in the middle of band 1, the double scute near the middle of band 6 and the scute in the middle of the left half of band 1 are varied manifestations of the same anomaly.

3. There is more extensive doubling in the two primary bud individuals II. and IV. than in the two secondary bud individuals I. and III.



Set K. 4, ♂ (Fig. 11).

The mother has a short series of 3 double scutes in positions 8, 9 and 10 from the left.

Fetus I. has a series of 5 double scutes occupying positions 5, 6, 7, 8 and 9 from the middle of the band.

Fetus II. has on the left 4 single scutes, and the rest of the

band, beginning in position 5, is double. The symmetry is that of the mother, but the doubling involves most of the band. The symmetry is a half-band reversal of that in fetus I. in that the doubling begins 5 scutes from the left margin instead of 5 scutes from the middle.

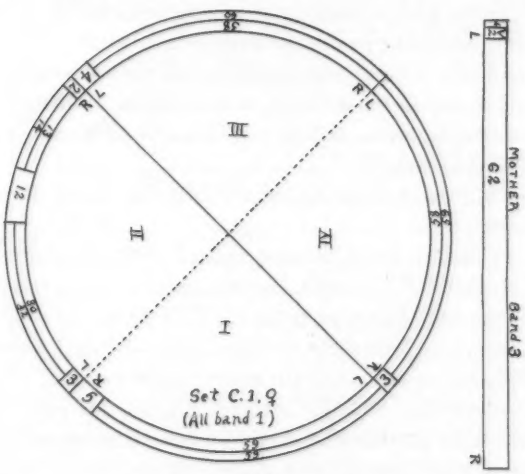
Fetus III. has either no anomaly at all or else has the entire band doubled. It is difficult to decide as to these alternatives.

Fetus IV. has the anomaly bilaterally symmetrically. One might claim that the anomaly that should have appeared on fetus III. has been transferred to fetus IV. so that the latter has a double dose of it.

Set C. 1, ♀ (Fig. 12).

The mother has in band 3 in the second position from the left a double scute.

Fetus I. has an extensive doubling beginning 5 scutes from the



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right-hand margin of band 1. Thus the symmetry of the mother is reversed.

Fetus II. has an extensive unequal bilateral doubling beginning after two scutes on the right and three on the left, continuing to the middle on the left and for 13–14 scutes on the right.

Fetus III. has the doubling of band 1, beginning 4 scutes from the left. The symmetry is the same as that of the mother, but a reversal of that of the opposite individual, fetus I. Fetus III. is therefore a mirror-image of fetus I.

Fetus IV. has the anomaly beginning after 3 single scutes from the right margin of band 1. The symmetry is a reversal of that seen in its twin partner, fetus III., and of that of the mother, so that III. and IV. are mirror-images of each other.

This set shows clearly that a double scute is genetically equivalent to a double band. It is seldom that one finds a set showing such extensive reversals of symmetry.

Set C. 29, ♂ (Table B1).

This set is the complement of set C. 1, in that the mother has an extensive band doubling and the offspring inherit it in the form of scute doubling.

The mother has in band 1 beginning 4 scutes from the left a doubling extending to the right margin.

Fetus II. has in band 7 the second scute from the right double.

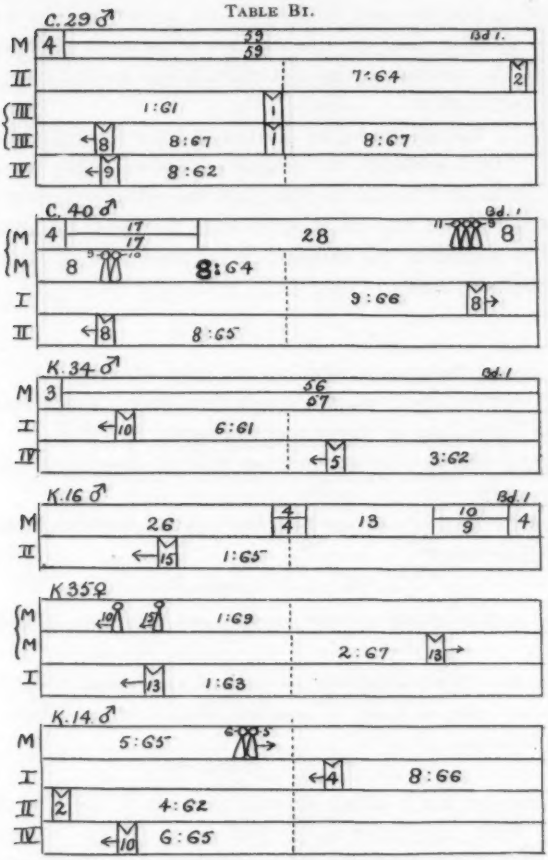
Fetus III. has in band 1 and in band 8 just to the left of the middle a double scute, and in addition a double scute 8 scutes from the left in band 8.

Fetus IV. has in band 3 a double scute in the ninth position from the left.

This set illustrates an extreme case of positional shifting of a genetic character. There can be no question of the relationship between the two scutes of fetus III., occurring in band 1 and band 8 just to the left of the middle. This is a case of reduplication of inheritance unit along the antero-posterior axis. Similarly scute 8 in band 8 of fetus III. and scute 9 in band 3 of fetus IV., are expressions at different levels of the antero-posterian axis of the same genetic unit.

The double element next to the right-hand margin of band 7 in fetus II. is certainly the same genetic unit as the element next to the middle of band 8 of fetus III., but with a reversal of symmetry. Thus by the application of the principle of symmetry reversals and reduplications down the primary axis we can connect up even such apparently unrelated units as these.

TABLE BI.



Set C. 40 ♂ (Table BI.)

Here the same type of inheritance is seen as in set C. 29, but the genetic relations are much clearer, and involve no strain upon one's credulity.

The mother has in band 1 a bilateral doubling, beginning on the left after four single scutes and extending in a series of 17-17 double scutes and beginning on the right after 8 single scutes and continuing for a short series of 3-3 double scutes. In addition we find in band 8 a doubling strikingly like that of the right side

of band 1, but reversed to the left and involving only 2 scutes. This is another case of reduplication in anterior and posterior parts of the banded region, but there is also in this case a symmetric reversal.

Fetus I. has in band 9 in position 8 from the right a double scute. This is evidently the genetic equivalent of the two double scutes in band 8 of the mother, but the symmetry is reversed.

Fetus II. has in band 8, 8 scutes from the right, a double scute which is doubtless the reversal or mirror-image of that in its twin partner, fetus I.

Set K. 34, ♂ (*Table B1*).

In this set the genetic relations are quite obscure.

The mother has in band 1, beginning after 3 single scutes on the left a doubling of the rest of the band.

Fetus I. has in position 10 from the left margin of band 6 a double scute.

Fetus IV. has in position 5 to the right of the middle of band 3 a double scute.

Set K. 16 ♂ (*Table B1*).

The mother shows a right lateral and a median doubling of band 1.

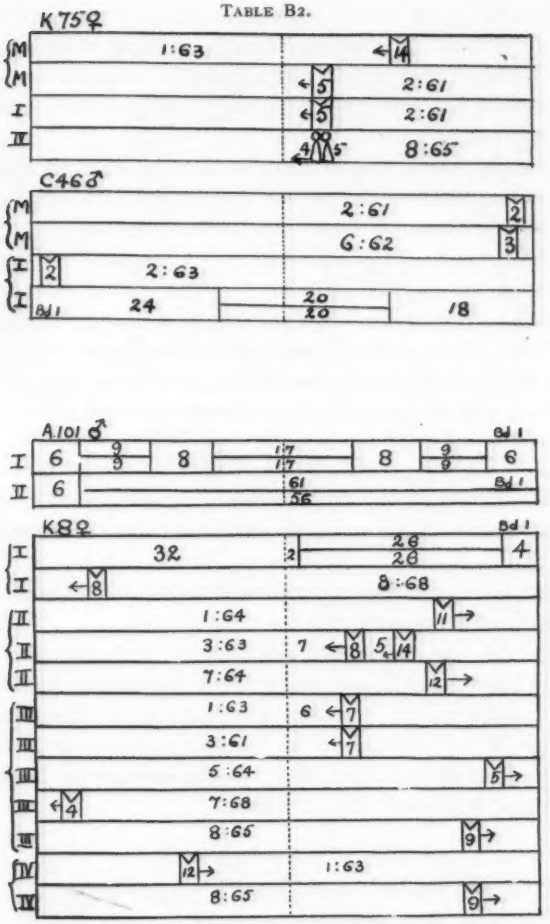
Fetus II. has one double scute in band 1, situated in the middle of the left side of band 1, another case of centro-lateral symmetry reversal.

Set K. 35, ♀ (*Table B1*).

The mother has a rather unusual anomaly in the form of two "split" scutes separated by several single scutes, occurring in band 1, in places 10 and 15 respectively from the left-hand margin. In band 2 we find the bilateral equivalent of these elements in the form of one double scute situated in position 13 from the right.

Fetus I. is the only one of the offspring to inherit the maternal anomaly. There is in band 1 a double scute situated 13 places from the left in the reverse position of that seen in band 2 of the mother. The genetic relations here are quite clear, but why should only one out of four offspring show an inherited character?

TABLE B2.



Set K. 14 ♂ (Table B1).

The mother has an incipient double band of two scutes in positions 5 and 6 to the left of the middle of band 5.

Fetus I. has a double scute in position 4 to the right of the middle of band 8. This shows reversed symmetry and a shifting down the primary axis of the genetic unit inherited from the mother.

Fetus II. has a double scute in position 2 from the left-hand margin of band 3. This is evidently a reversal of the conditions seen in the mother and is also a complex reversal, with shifted position on the primary axis, of the condition seen in its twin partner, fetus I.

Fetus IV. has a double scute in band 6, situated with reference to the middle of the left half band in about the same relation as is the peculiarity of the mother with reference to the middle of the whole band. In other words it is 5 scutes to the left of the middle of the left half of band 6, while that of the mother is situated 5 scutes to the left of the middle of band 5. Considering the rarity of anomalies of any sort in the bands 3, 4, 5 and 6, there can be no doubt of the genetic identity of these elements. Granting this the interpretation offered is not forced.

Set K. 75, ♀ (*Table B2*).

Here the mother has two double scutes, one near the middle of the right half of band 1 and another to the right of the middle of band 2.

Fetus I. has a double scute in band 2 identical in position with one of those of the mother.

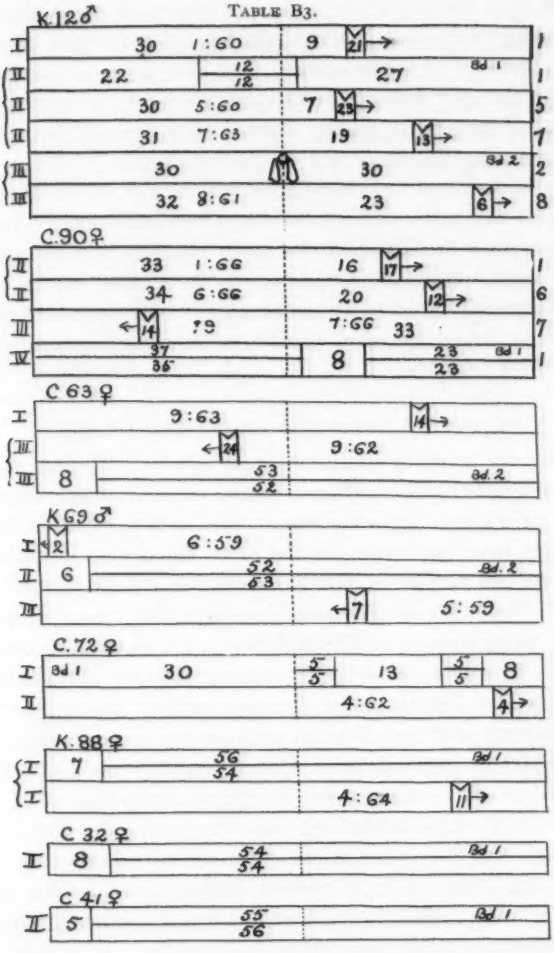
Fetus IV. has in band 8, in a position with reference to the middle identical with one of those of the mother and that of fetus I., an incipient band doubling of 2 scutes. This is another example of the shifting of an anterior element to a position further down the primary axis. It also emphasizes the genetic identity of band and scute doublings.

Set C. 46, ♂ (*Table B2*).

The mother shows a reduplicated double scute anomaly in bands 2 and 6 in positions 2 and 3 respectively from the right-hand margin.

Fetus I. shows in band 2 an exactly reversed mirror-image reversal of the anomaly in band 2 of the mother and in addition an extensive doubling in the middle of band 1. This illustrates three points; (*a*) reduplication down the primary axis, (*b*) the genetic relation between scute and band doubling, (*c*) the half-band type of reversal of symmetry.

TABLE B3.



It should be noted by way of summary that in all cases in which the mother has a band anomaly, one or more offspring of a set have usually a similar band anomaly; but sometimes a scute anomaly is found to be the expression in the offspring of a maternal band anomaly. In several cases where there are band anomalies in the offspring a scute anomaly is found in the mother.

It has been shown clearly in several sets of offspring that there is an extremely close genetic relation between band anomalies and anomalies of single scutes. The two types of anomaly are merely more or less extensive expressions of the same genetic factor. Whenever a mother shows either type of anomaly, one or more offspring show one or the other type. The doubling factor is therefore strikingly dominant in the Mendelian sense. When we find sets of fetuses that show anomalies and the mothers of these show no anomalies, we must conclude that the anomaly is paternal, for a dominant factor in the mother, would appear phenotypically if present genotypically. It is interesting to note that almost the same number of sets have normal as have anomalous mothers.

Anomalous Offspring of Normal Mothers.

There are in the present collection 14 sets of fetuses showing band anomalies, the mothers of which showed neither band nor scute anomalies. In some ways these sets are better for our purposes than are those derived from anomalous mothers, because we can be sure of the uniparental character of the inheritance. Since the mothers show no anomalies, those in the offspring, being unquestionably inherited, must have come from the father. It would be highly interesting to know the conditions in the fathers, but the latter are inaccessible. Since there is no sex difference with regard to the anomalies, we may assume, however, that the same state of affairs would be revealed for the paternal as that made out for the maternal relation.

Some of the most interesting cases illustrating the symmetrical distribution of anomalies, intra- and inter-individually, are found among the offspring from normal mothers, and I shall give a complete tabulation of these sets, calling attention in the text only to certain of the more striking conditions, or to situations differing from those dealt with in the sets derived from anomalous mothers.

Set K. 2, ♀ (*Fig. 13*).

This set perhaps better than any other shows the phenomenon of symmetry reversal, or mirror-imaging among the quadruplets.

Fetuses I. and III., which face one another across the vesicle,

and are the secondary bud derivatives of II. and IV. respectively, are exact symmetrical reversals of each other, I. having the anomaly only on the left and III. only on the right.

Fetuses II. and IV., the two primary bud individuals, both show the anomaly bilaterally, but are also reversals, in that II. has the more extensive doubling 9–9 on the right and IV. has the

13

more extensive doubling 9–8 on the left. It is interesting to note that the primary individuals II. and IV. are bilateral in their expression of the anomaly, and that the secondary individuals I. and III. are unilateral.

Set A. 64, ♂ (Fig. 14).

This set is remarkable for the striking identity of detail exhibited by the anomalies of the different fetuses.

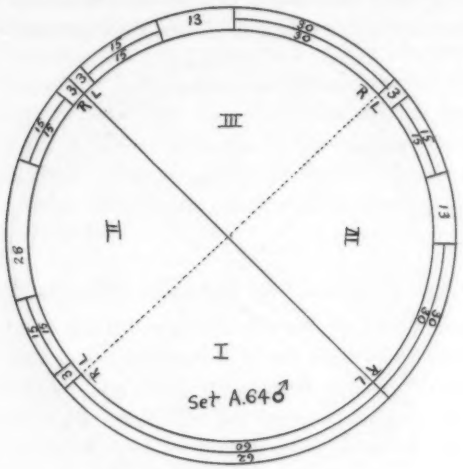
Fetus I. has both halves of band 1 completely double.

Fetus II. has both halves partially double and exactly bilaterally symmetrical, the doubling starting 3 scutes from the margin on each side and extending for 15–15 scutes.

Fetuses III. and IV are both exactly like II. on the left side and exactly like I. on the right.

There is exactly the same amount of doubling in I. plus II.

as there is in III. plus IV., but the distribution of the fully doubled and incompletely doubled half bands is different. In the case of the left-hand pair I. and II., I. gets both completely doubled halves and II. gets both incompletely doubled halves;



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while in the case of III. and IV. both get a completely and an incompletely doubled half. There is no symmetry reversal in this set.

Set A. 96, ♂ (Fig. 15).

Fetus I. has band 1 double except for 5 single scutes on the right margin.

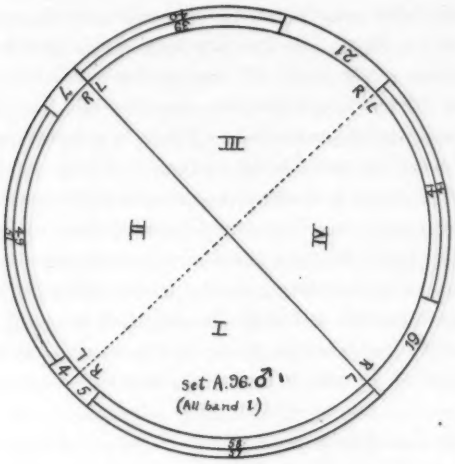
Fetus II. has a similar anomaly bilaterally.

Fetuses III. and IV. are very much alike but differ from I. and II. in the extent of the single part of the band, which is much longer than in the other pair. The pairing of fetuses is very clear, but no symmetry reversal is shown. One of four fetuses (II.) is bilateral.

Set K. 80, ♂ (Fig. 16).

Fetuses I. and II. are practically identical. In both band 1 is doubled, except for four single scutes on the left.

Fetuses III. and IV. show the incomplete doubling bilaterally



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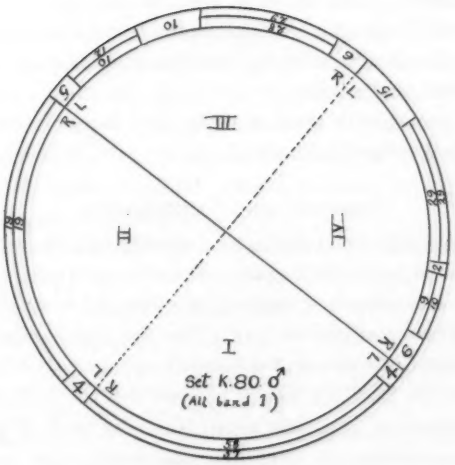

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with a larger and smaller series of double scutes arranged on opposite sides. Fetus III. has the long series 28–29 six scutes from the right, while fetus IV. has a series of 29–29 scutes 15 scutes from the left and extending past the middle. This is an imperfect reversal of symmetry. There is a better reversal in connection with the short series of double scutes, for in III. the series of 10–12 scutes is situated 5 scutes from the left, and in IV. the series of 9–10 scutes is situated 6 scutes from the right.

Set A. 101 (Table B2) has the anomaly confined to one pair of fetuses (I. and II.) and the expression is very different in the two. It is well to note that although the doubling is nearly complete in II. it starts after 6 single scutes at the margin, as is the case on both sides of I., which shows a remarkably exact bilateral symmetry.

In the remaining sets, K. 8, K. 12, C. 90, C. 63, C. 69, C. 72, C. 88, C. 32 and C. 41 (Table B2 and 3), band doubling is found in only one fetus of a set. In most sets however scute anomalies, which are usually rather definitely related to the band anomaly, are present in one or more fetuses. Set K. 8 is remarkable for the large amount of reduplication down the axis of scute anomalies, for example fetus III. has double scutes in bands 1, 3, 5, 7, and 8, those on bands 5 and 7 being reversals of each other. It is clear that all of the double scutes are in the same region of the band as is the double series in fetus I., and the majority are on the same side of the body.

The remaining sets show nothing that has not already been seen in sets previously described.

Summary and Conclusions.

Band anomalies are of the nature of irregularities in the normal regular rows of scutes that make up the typical band, and consist of more or less extensive regional doubling of rows of scutes in a band. This condition is typical for the non-banded parts of the armor but quite rare in the banded regions. It is practically confined to the band or bands nearest the non-banded region. Band anomalies occur in only about three per cent. of individuals and an examination of over two thousand adult individuals taken at random shows no duplicate anomalies.

These anomalies are strongly inherited but are subject to more or less modification. Sometimes a band anomaly unilaterally placed may be inherited unilaterally but with a reversed symmetry to that of the mother, or it may be bilateral. Sometimes an extensive doubling is inherited both as a similar band doubling, and as a single double scute in the individuals of a single polyembryonic set of offspring. Contrariwise a double scute in the mother may be inherited as a more or less extensive unilateral or bilateral band doubling. The peculiarity may also be reduplicated down the primary axis in two or more bands.

Frequently in unilateral anomalies the different fetuses of a set show reversed symmetry or mirror-imaging, but it is even more common to find the unilateral anomaly on the same side of most of the individuals, or bilaterally in one or more of them.

In a number of cases the anomalies in different fetuses of a set are so strikingly identical as to indicate a rigid predetermination of the details of the character, but in other cases there appears to be only a predetermination of a generalized anomaly that expresses itself to a greater or less extent in the various embryos. In terms of Mendelian inheritance we may say that an anomaly factor is inherited as a dominant character, but its distribution among the fetuses of a set and its location and extent are due to varying ontogenetic or epigenetic factors.

The distribution of these inherited anomalies among the various fetuses of the sets furnishes much interesting data, which, together with data on the inheritance of double scutes, to be presented subsequently, furnishes the basis of a discussion of several general questions and especially those involved in the concept of organic symmetry.

This subject is reserved for a subsequent paper of the present series.

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FACTORS CONCERNED IN THE PRODUCTION OF MITOSIS IN ORGANISMS DISPLAYING CELL CONSTANCY.[1]

H. J. VAN CLEAVE.

The condition of absolute identity in cellular structure found in individuals of many species of Metazoa has led the writer to undertake an analysis of the factors governing mitosis in these forms. Loeb ('12: 4) has called attention to the fact that the first attempt to reduce the phenomena characteristic of life to purely physico-chemical terms is found in the works of Lavoisier and Laplace ('80) which indicated that the heat produced in the body of a warm-blooded animal equalled that given off by a burning candle when the amounts of carbon dioxide produced in the two instances were equal. These results have been so much amplified by later workers that today no one doubts that the general processes having to do with the phenomena of metabolism are identical with the so-called purely physical and chemical reactions occurring outside the living body. In fact while metabolism, movement, and irritability are generally granted as probably due to the workings of the same principles that govern the inanimate realm the fourth of the vital properties, that of reproduction, is in great part still unexplained. No problem has offered more secure harbor for a final possibility of the presence of some supernatural force than has this one dealing with the processes of reproduction. The statement of Davenport ('08: 1); "The vital processes are chemical processes, taking place in a highly complex, very unstable, constantly changing substance, whose activities we call life," embodies the modern conception of the nature of life phenomena in general, but no attempt has been made to show the actual possibility of explaining all life processes on this basis.

An analysis of the possible factors determining or directing

[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 43.

the course of nuclear and cell division is extremely difficult on account of problems involved in eliminating factors until but a single causal agent is operative. However, of the possible factors involved in the production of mitosis and of cleavage two groups are clearly recognizable, namely: (1) Environmental factors, and (2) Internal factors. Any change which occurs within the living organism must find explanation upon the basis of one of these two groups of factors or upon the basis of a combination of them. It is the purpose of this paper to show the relationship of these two groups of factors in the production of mitosis among those organisms which are made up of a fixed number of cells.

1. Environmental Factors.

If environmental factors either caused or directly controlled the phenomena of mitosis the fact of cell constancy could not exist, for no two individuals, and more strongly no two groups of individuals even of the same species, develop under absolutely the same environmental conditions. Consequently if environmental factors were the limiting factors in mitosis no two individuals would of necessity contain the same number of cells. It is generally granted that the mitotic process may be accelerated or retarded through the application of purely external stimuli. At least in those organisms made up of a fixed number of somatic cells such an acceleration or retardation of the mitotic process could result in nothing beyond a modification of the normal *rate of the process* and could in no wise be considered as a direct factor in the determination of the *extent of the series* of mitotic divisions during the cleavage stages and in subsequent development. If the role of temperature, for instance, be examined as a possible controlling factor in the mitotic process it becomes apparent that if this alone and directly controlled the number of mitotic divisions through which the developing organism should pass there could be but slight possibility of any two individuals having identical numbers of somatic cells. For under conditions of nature no two individuals are at all times during their development under absolutely identical temperature relations. A similar variability of conditions for the development of different individuals exists in the case of almost all of the other environmental

factors which might be considered as directly influencing the process of mitosis.

Furthermore if environmental conditions could directly control and determine the mitotic process, cell division would continue indefinitely through the life of the organism as long as the external conditions remained favorable for it. Again the data brought from the field of cell-constancy show the impossibility of such factors being operative in a controlling manner. In the development of any organism or part of organism the powers of reproduction of most of the somatic cells are restricted to definite periods in the early stages of development, usually preceding the introduction of histological differentiation. Thus in the genus *Eorhynchus* the writer ('14: 280) has shown that in no instance did he find any adult worm displaying abnormal numbers of somatic nuclei or presenting any evidence whatever of further division of the somatic nuclei after the adult body form had been attained. These observations were upon over two hundred individuals collected during a period of four years at various localities and at various seasons of the year.

In conclusion, if the determination of the mitotic process were to find its explanation in terms of environmental factors, or to a continuous production of combinations of environmental factors during the process of development, then in every instance the number of mitotic divisions a fertilized egg would undergo would be a direct resultant of the complex of environmental factors operative during its process of development. At least in those forms with a high degree of cell constancy it seems obvious that purely environmental factors have but one relationship to the process of mitosis and that consists in the modifiability of the rate, either as an acceleration or as a retardation. In this respect purely environmental factors have the same general effect upon the process of mitosis as they exert upon purely physical and chemical reactions.

2. Internal Factors.

Our incomplete knowledge of the finer structure of the cell, and of the nucleus in particular, make it impossible to associate the control of the mitotic process directly with any structure or

chemical bodies found in the cell. However on the basis of facts pointed out earlier in this paper it seems certain that since there is no possibility of environmental factors acting as the controlling element in mitosis the ultimate cause of the process must be sought within the cell. In this connection invaluable support is found in the field of experimental embryology. Morgan ('95, '01, and '03) and Driesch ('98 and '00) have both shown that in the embryos of echinoderms developed from isolated blastomeres of the two cell stage the number of cells present at any point in the development is approximately half of the number present in a normal embryo. Similarly from one of the blastomeres of the eight cell stage the gastrula is composed of only one eighth the number of cells found in the normal gastrula. Loeb ('06: 59) interprets these results as supporting the hypothesis of Sachs ('93 and '95) which regards the factors determining cleavage controlled by the ability of each nucleus to gather around itself and control a definite amount of protoplasm. Yet what determines the amount of protoplasm present in the developing individual? The cytoplasm is constantly being replaced through the processes of anabolism which experiments with enucleated cells have shown to be under the control of the nucleus. Consequently it seems that the view just stated comes not much short of being an argument in a circle. Does the amount of cytoplasm determine the number of nuclei that are to be formed or is the numerical relation of nuclei to cytoplasm a mutual one brought about not through the influence of either cytoplasm or of nucleus but through some fundamental factor which determines the number of nuclei and at the same time indirectly the amount of cytoplasm that is to be formed? The writer interprets the data of Morgan and of Driesch in an entirely different manner. If by the first division of the egg there are set apart two units, each of which has the possibility of developing into a given number of cells by the process of mitosis and this tendency is retained, even though the two units become separated, it seems logical to conclude that within the fertilized egg there are resident potencies which through the process of mitosis become divided between the two daughter nuclei of the first and then of each succeeding generation of cells.

As to the nature of this partition with each mitotic division two explanations present themselves. According to the simplest of these mitosis may be the result of the direct chemical activity of certain substances, present in the fertilized egg, which become used up in the mitotic process so that each cell of the two cell stage receives an equal amount of the substance present in the fertilized egg after the amount necessary for the first mitotic division has been eliminated. On this hypothesis with each succeeding mitotic division the materials resident in the developing embryo become partitioned and dissipated in the process of development. Thus if x equals the entire amount of the material for the execution of the mitotic process present in the fertilized egg, and a the amount required for the realization of the first mitotic division then each cell of the first cleavage would receive $(x - a)/2$. In this manner each succeeding division would reduce the amount of the material present until the amount apportioned to each cell would be less than the amount required for the execution of the mitotic process, thus bringing about an automatic check upon the course of the series of nuclear divisions. The following objection to such a hypothesis shows its weak point. Upon this basis each blastomere of the early stages would be required to produce the same number of cells, a supposition which the facts of cell lineage do not support.

The second explanation of the method of control over the number of divisions of the nucleus seems more natural and does not convey so much of the idea of predestination, in that it requires less emphasis upon the inherent qualities of the fertilized egg. According to this hypothesis mitosis is just as markedly a result of chemical processes going on within the egg as indicated above but the individual cells may in varying degrees retain the power of synthesizing the materials necessary to initiate and carry out the mitotic division of the nucleus. In the application of this explanation the check to the course of the nuclear divisions may come as the result of an accumulation of materials within the cell, probably as metabolic by products, which serve to retard and finally to prohibit the chemical activity incident to nuclear division. Thus at the end of any definite period of physiological activity the organism will be composed of a definite

number of cells and so upon attaining maturity will have a fixed number of somatic cells which are unable to divide farther on account of the presence of the inhibiting materials. It must be remembered that throughout this discussion principles are being laid down for the determination of the mitotic process in organisms or parts of organisms displaying cell constancy. The objection involving the variability in the number of cells arising from the early blastomeres, cited in connection with the discussion of the influence of environmental factors, finds no grounds here. In fact it lends its support to this second hypothesis. If the ultimate check to the mitotic process comes as the result of an accumulation of metabolic by products, then the *rate* of the nuclear division, which unquestionably may be influenced by environmental factors, would tend but to be directly proportional to the rate of the accumulation of the inhibiting elements, though the actual amount of the inhibitors necessary to terminate the series of mitotic divisions would remain the same. Evidently the accumulation of the inhibitors proceeds unequally in various tissues of the metazoan body. The degree of the differentiation of the cell and, in all probability connected with this, the nature of the cell membranes, determines the rate of the accumulation of the inhibiting factors.

The impossibility of explaining the phenomena of cell constancy on the basis of mitotic control by environmental factors and the facts from experimental embryology in their bearing upon the control of mitotic division by internal factors, lead to the evident conclusion that during the development of cell constant forms the mitotic process is controlled and determined from within the cell but its rate may be regulated by factors which are considered purely environmental.

Conclusions.

1. At least in those forms displaying a high degree of uniformity in the number and arrangement of their component cells and nuclei, environmental factors such as ordinarily influence physical and chemical changes play no direct part in the determination of the number of mitotic divisions of the somatic nuclei.

2. The only influence of normal environmental factors in these

forms consists in the modification of the rate of the process of mitosis.

3. The direct control of the mitotic process must be sought in the chemical activity going on within the cells of the developing individual.

4. Experimental evidence indicates that with each cleavage of an egg with determinate cleavage there is retained a definite relationship between the number of any given cleavage and the total number of cleavages that the embryo would undergo even in those cases where the blastomeres become isolated in the early stages of development.

5. The foregoing would indicate clearly that within the cells derived from the fertilized egg there are present factors or potencies which exert direct control over the number of mitotic divisions which shall ensue.

6. The fact that not all of the blastomeres of the early cleavages produce the same number of cells indicates that the number of cells produced must be controlled by conditions developing as the process of development progresses rather than by the partition and distribution of some definite materials present in the egg at a time prior to the first cleavage.

7. In tissues which retain the power of continued mitotic division, as for example in the formation of the germ cells and in tissues which have widely varying numbers of cells, the explanation of the inconstant nature of the numbers of cells produced may be sought in the acquisition of the power of eliminating from the cell those materials which in the course of the process of metabolism tend to accumulate and serve as inhibitors to the mitotic process.

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THE PRODUCTION OF MALES AND FEMALES CONTROLLED BY FOOD CONDITIONS IN THE ENGLISH HYDATINA SENTA.

DAVID D. WHITNEY.

It has been found recently in an American parthenogenetic strain of the rotifer, *Hydatina senta*, from New Jersey that a continuous diet of the colorless protozoan flagellate, *Polytoma*, causes practically all females to be produced.[1] This production of only females can be maintained in this manner through generation after generation for several years. If, however, the diet is suddenly changed to a green protozoan flagellate, *Chlamydomonas*, that is in an active state, males can be produced in great numbers.

It has been suggested that perhaps this phenomenon of the regulation of the two sexes by food conditions is peculiar to this particular strain of New Jersey *Hydatina senta* and is not an universal characteristic of the species. Fortunately it has been possible to test this hypothesis on an English strain of *Hydatina senta* and some very clear and conclusive results have been obtained.

I am greatly indebted to A. F. Shull for the stock of English rotifers with the accompanying data. "The English line was received from Mr. C. F. Rousselet, who collected them as resting eggs in mud at the bottom of a duck pond in England in August, 1912. They were sent to me about November 1, 1912, in dry dirt. The first ones hatched a few days later and from them the line sent to you has been reared by parthenogenesis ever since. As they were reared there was a generation about every three days and so you have about the 270th generation." The stock of this English line was received from Dr. Shull on January 7, 1915.

The females of this strain produce fewer offspring in the same period of time than the females of the New Jersey strain. This

[1] *Jour. Exper. Zool.*, Vol. 17, November, 1914.

is probably due to the fact that the former strain had been reproducing parthenogenetically for several years and had become weakened whereas the latter strain was developed from a resting egg in November, 1914, and at the present time is as vigorous as at first.

The method of making the media and rearing the two kinds of protozoan food cultures has been given in detail in a former paper and will not be repeated here, excepting a few additional words about the green food. When the *Chlamydomonas* had been 2–3 weeks in the same media the individuals were large and more or less quiescent. In this condition they were only moderately effective although in one or two instances some that had been two to three months in the same media were very effective. When they had been in new bouillon media for 2–7 days with considerable sunshine at a temperature of about 28° C. they were of various sizes and seemed to be the most effective. However, there seems to be a considerable amount of chance in getting the *Chlamydomonas* in the optimum condition for every experiment. This probably explains the varying percentages of the male-producing females obtained in the different experiments.

As it seems that the effect of a uniform diet on this rotifer has not been sufficiently emphasized the following Table (I.) concerning this point in three pedigreed races has been compiled from the records in an earlier paper.[1] This shows that a constant and uniform diet of *Polytoma* has repressed the production of males for nearly 300 generations. The long period of time

TABLE I.

SHOWING THAT A CONSTANT AND UNIFORM DIET OF THE COLORLESS PROTOZOA, *Polytoma*, REPRESSES THE PRODUCTION OF MALE-PRODUCING FEMALES AND CAUSES THE PRODUCTION OF FEMALE-PRODUCING FEMALES.

Kept on a Uniform Polytoma Diet.

Race.	Generation.	♂♀	♀♀	♀♀	Time.
A.....	1–288	92	2,565	96+	22 months
B.....	1–181	8	1,731	99+	14 months
C.....	1–288	0	2,749	100	22 months

[1] BIOL. BULL., Vol. 22, March, 1912.

and the large number of generations through which these races were reared and observed would seem to warrant the reliability of the results.

Table II. contains the results of some new observations made in November and December of 1914. The main point of interest in it is that it shows at what place or stage after the diet has been changed females may be isolated that will produce a high percentage of male-producing daughters. Adult females (mothers) that were put into the new diet of green food produced in it,

TABLE II.

AS A CONTROL FOR TABLE III. AND ALSO SHOWING THE HIGH PERCENTAGE OF MALE-PRODUCING DAUGHTERS PRODUCED AFTER THEIR MOTHERS HAD BEEN TRANSFERRED FROM THE *Polytoma* DIET TO THE GREEN *Chlamydomonas* DIET FOR 12 HOURS.

Experiments During November and December 1914 on a New Jersey Strain of Hydatina senta.

Experiment.	Control Reared and Continued on a *Polytoma* Diet.				Adult ♀♀s from the Control Reared on a *Polytoma* Diet Transferred to a *Chlamydomonas* Diet.						
	♀♀ Mothers.	Daughters.			♀♀ Mothers.	Daughters Produced During First 12 Hours.			Daughters Produced During Hours 12–24.		
		♀♀	♂♀	⚥♂♀		♀♀	♂♀	⚥♂♀	♀♀	♂♀	⚥♂♀
1	10	10	0	0	6	14	20	59+	0	22	100
2	10	10	0	0	5	33	6	15+	14	12	46+
3	10	10	0	0	5	24	6	20	8	32	80
4	10	10	0	0	5	No record			7	17	70
5	10	10	0	0	10	"	"		31	61	66*
6	10	10	0	0	5	7	13	61+			
7	10	10	0	0	40	No record			9	104	92+
8	10	10	0	0	40	"	"		24	126	84
9	10	10	0	0	40	"	"		30	129	81+
10	10	10	0	0	10	"	"		1	39	97+
	Control continued through 30 additional generations										
	300	300	0	0							
Total....	400	400	0	0	166	78	45	36+	124	542	81+

during 1–12 hours, 15 per cent.–60 per cent. of male-producing daughters, but the same adult females in the second 12 hours produced a much higher proportion of male-producing daughters, usually from 80 per cent.–100 per cent. In most of the experiments the adult females were taken out of the green food after they had been in it 12 hours, in more or less of sunlight, and placed in filtered culture water from a general stock battery jar

in which various protozoa and rotifers were living. They were left in this filtered water for about 12 hours, during the night, without food. During this time each female laid 3–4 eggs and the next morning the old females were taken out, *Polytoma* food was added, and the eggs allowed to hatch. Several hours later the young females that hatched from these eggs were isolated in separate watch glasses and fed *Polytoma* and a species of a small *Euglena*.

In Table III. this same fact is shown again to be true for the English species. However, this is a relatively minor point and

TABLE III.

SHOWING THAT WHEN THE ENGLISH STRAIN WAS SUBJECTED TO A UNIFORM AND CONSTANT DIET OF THE COLORLESS PROTOZOA, *Polytoma*, ONLY FEMALE-PRODUCING FEMALES WERE PRODUCED BUT THAT WHEN IT WAS SUBJECTED TO A SUDDEN CHANGE OF DIET FROM THE *Polytoma* TO A GREEN PROTOZOA, *Chlamydomonas*, AS HIGH AS 85 PER CENT. OF MALE-PRODUCING FEMALES WAS PRODUCED.

Experiments During January and February 1915 on an English Strain of Hydatina senta.

Experiment.	Control Reared and Continued on a *Polytoma* Diet.				Adult ♀♀s from the Control Reared on a *Polytoma* Diet Transferred to a *Chlamydomonas* Diet.						
	♀♀ Mothers.	Daughters.			♀♀ Mothers.	Daughters Produced During First 12 Hours.			Daughters Produced During Hours 12–24.		
		♀♀	♂♀	⅘ ♂♀		♀♀	♂♀	⅘ ♂♀	♀♀	♂♀	⅘ ♂♀
1	10	10	0	0	5	7	4	36+	4	14	77+
2	10	10	0	0	5	8	2	20	5	5	50
3	10	10	0	0	5	4	3	42+	9	3	25
4	10	10	0	0	5	14	4	22+	8	4	33+
5	10	10	0	0	20	29	11	27+	16	27	62+
6	10	10	0	0	20	38	8	17+	7	40	85+
7	10	10	0	0	15	No record			15	13	46+
8	10	10	0	0	20	30	18	37+	No record		
9	10	10	0	0	90	No record			50	76	60+
	Control continued through 20 additional generations										
	200	200	0	0							
Total....	290	290	0	0	185	130	50	27+	114	182	61+

may only be of importance to any one wishing to obtain cytological material.

If Table III. is compared further with Table II. it is evident that they are very similar in all points with the exception that

the percentages of male-producing females in Table III. are somewhat lower than those in Table II. However, both tables show that a continuous and uniform diet of *Polytoma* causes only female-producing daughters to be produced while a sudden change of the food from *Polytoma* to *Chlamydomonas* causes male-producing daughters to be produced in great numbers.

Summary.

1. A uniform diet of the colorless protozoan flagellate, *Polytoma*, continued for 22 months through 288 generations practically suppressed the production of males and caused only females to be produced in the rotifer, *Hydatina senta*.

2. After the adult females had been transferred from the diet of *Polytoma* to the diet of *Chlamydomonas* for 12 hours they produced a higher percentage of male-producing daughters than they did during these 12 hours.

3. The sudden change of food from a constant diet of *Polytoma* to a diet of the green *Chlamydomonas* caused male-producing daughters to be produced in the English *Hydatina senta* about as readily as in the New Jersey *Hydatina senta*.

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MICROTÆNIELLA CLYMENELLÆ, A NEW GENUS AND NEW SPECIES OF COLONIAL GREGARINES.

GARY N. CALKINS.

While examining the gut contents of marine annelids at Woods Hole during the month of June, 1914, a number of new gregarines were discovered. The majority of these were ordinary types which could not be placed systematically without knowledge of the sporulation stages. One form, however, found in the digestive tract of *Clymenella torquata*, deserves mention because of its remarkable novelty.

To obtain the material, the worms were opened along the mid-dorsal line; sections of the digestive tract about half an inch long, were removed and teased in salt solution on cover glasses. The material thus prepared was examined while fresh, and, if interesting, was then fixed in sublimate acetic and stained. In this way I have obtained more than a hundred specimens of the curious organism described here.

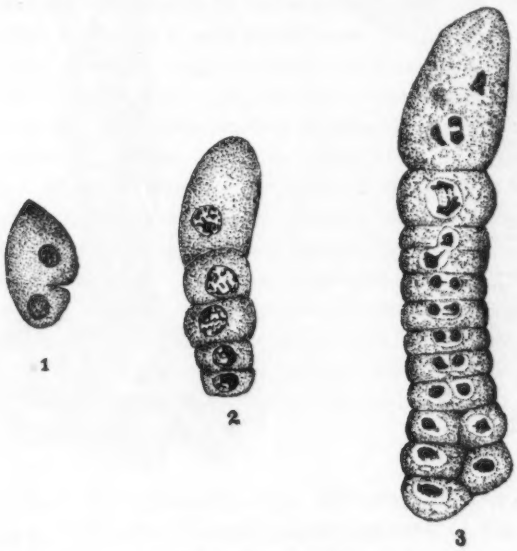
The name *Microtæniella* is given because of the Tænia-like structure of the organism (Figs. 1–5). The initial single cell resembles a scolex and the chain of cells, formed partly from the initial cell and partly by cell division of the daughter individuals, resembles a chain of proglottids. In sporozoan terminology these may be termed the primite and satellites respectively, although this involves some elasticity in the use of the terms primite and satellite which are usually employed to designate the primary and secondary individuals in a chain of gregarines formed by secondary association.

In the living state, the organism is colorless but with the characteristic dense protoplasmic structure of the gregarines. The average dimensions of the chain are 84 μ in length and 15 μ in width, the size of mature chains varying but little from the average.

The earliest stage found was an initial cell with two nuclei and the beginnings of a septum cutting off a first satellite (Fig. 1).

All intermediate stages between this young form and stages pictured in Figs. 4 and 5 were found. Each satellite contains a single nucleus except in the division stages and in the terminal cells of the oldest satellites.

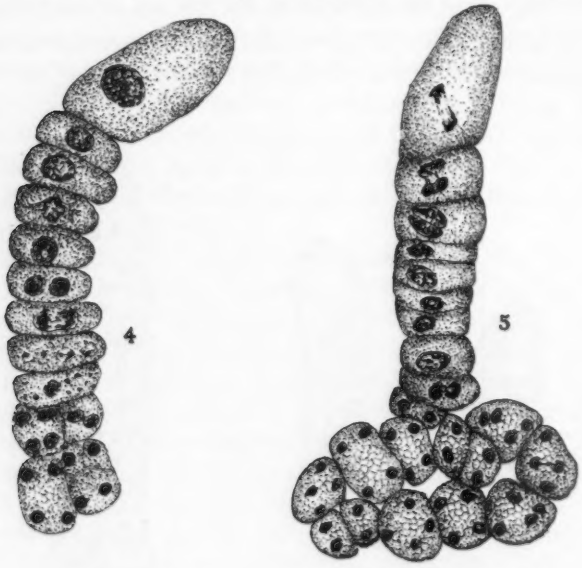
New cells are continuously given off by the primite; these reproduce by division, the plane of division being at right angles to the long axis of the aggregate for the first one or two divisions. In some chains the successive individuals furnish almost every stage for an ideal diagram of cell division (Fig. 3). In the older



satellites the plane of division changes from transverse to longitudinal in respect to the long axis of the chain. In this way two terminal chains may originate, but such branch chains never become very long owing to further development and to separation of the satellites from the parent colony.

After from eight to ten satellites have been produced thus by division, the terminal cells become more spherical, their nuclei divide twice, and without cytoplasmic division, giving rise to a terminal group of cells each with four nuclei (Fig. 4). This stage must persist for some time for several specimens were found

without evidence of further advance. In one specimen (Fig. 5), a group of terminal satellites, evidently the result of this phase of activity in two branched terminal chains, were present. Ultimately, division planes appear in the cytoplasm around each of the four nuclei, and four naked cells are produced. The fate of these cells is not known; possibly they become new primites



and repeat the cycle, but more probably, they are gametes which unite to form sporoblasts characteristic of the gregarines. I shall try to work out their further history during the coming June.

This rather paradoxical organism has given me a great deal of trouble. Is it a metazoön or a protozoön? Is the "individual" the entire chain or is it a chain of individuals? Although the aggregate has a definite morph and a definite ontogeny I am inclined to regard it as a chain of individuals in which original reproduction by division has become modified to asymmetrical division or budding in the primite, but is retained by the satellites. Originally, it may be assumed, the daughter individuals became separated and after a few divisions finally gave rise to

sporonts. If the entire chain is regarded as the individual we are met by the same difficulty which confronts us in connection with the colonial flagellates.

The possibility of this being a plant form has not been overlooked. The absence of definite walls together with the method of cell division and terminal cell changes are opposed to what is known of types in the group of fungi. To make sure, however, I showed the specimens to my colleague Professor R. A. Harper who gives me permission to quote him to the effect that he finds nothing in the structure or the history of this organism that would justify him in placing it with plant forms.

Amongst protozoa, nothing, so far as I am aware, has been described of like nature. Leger's *Tæniocystis* is a gregarine with numerous septa and with external annulations which give it the appearance of a *Tænia*, but it is a single cell and has a single nucleus. The absence of motile organs in the present form, and its mode of reproduction, leave no grounds for placing it otherwise than with the sporozoa, and here the only possible place for it is with the gregarines. There is some evidence that the often sharply pointed end of the primite is the attaching portion, but no stage showing such attachment has been found in the smears.

I would classify *Microtæniella* as a colonial parasite belonging to the order Gregarinida, suborder Schizogregarina, of which it should form a new subdivision.

COLUMBIA UNIVERSITY,
March, 1915.

THE BLINDNESS OF THE CAVE FAUNA AND THE ARTIFICIAL PRODUCTION OF BLIND FISH EMBRYOS BY HETEROGENEOUS HYBRIDIZATION AND BY LOW TEMPERATURES.[1]

(WITH 13 FIGURES.)

JACQUES LOEB.

I.

While many of the animals inhabiting caves are blind or have degenerated eyes, the same phenomenon is rarely found among animals that live in the open. At first sight this seems to suggest that the disuse of the eyes in the complete darkness of the cave has gradually led to the degeneration of the eyes and this idea seems at one time to have been widely accepted. In forming a judgment of the connection between the darkness of the caves and the blindness of cave dwellers we must remember that some of the cave dwellers have perfectly normal eyes. Thus Eigenmann, to whom we owe the most thorough study of this subject, points out that of the four species of salamanders living habitually in North American caves two have apparently quite normal eyes. They are *Spelerpes maculicauda* and *Spelerpes stejnegeri*. Two others living in caves have quite degenerate eyes, *Typhlotriton spelæus* and *Typhlomolge rathbuni*.[2] If disuse is the direct cause of blindness we must inquire why *Spelerpes* is not blind.

Another difficulty arises from the fact that a blind fish, *Typhlogobius*, is found in the open (on the coast of southern California) in shallow water, where it lives under rocks in holes occupied by shrimps. One must again ask the question: How can it happen that in spite of the exposure to light *Typhlogobius* is blind?

The most important fact is perhaps the one found by Eigen-

[1] From the Rockefeller Institute for Medical Research, New York.

[2] Eigenmann, "Cave Vertebrates of America," Carnegie Institution Publications, Washington, 1909.

mann in the fishes of the family of Amblyopsidæ. Six species of this group live permanently in caves and are not found in the open, while one lives permanently in the open and is never found in caves and one comes from subterranean springs. The one form which is only found in the open, *Chologaster cornutus*, has a simplified retina aside from having a comparatively small eye, in other words, its eye is not normal. This indicates the possibility that the other representatives which are only found in caves also might have abnormal eyes even if they never had lived in caves.

Through these facts the old idea becomes doubtful, namely, that the cave animals had originally been animals with normal eyes in which the disuse had led to a gradual hereditary degeneration of the eyes. Instead we must consider the possibility that in the blind cave animals as well as in the blind animals which live in the open the tendency towards blindness developed independently of presence or absence of light. From this point of view the tendency toward degeneration of the eye appears as a hereditary mutation comparable to the inherited glaucoma which is known in the human. Glaucoma is a form of blindness caused by the atrophy of the optic nerve in consequence of an increased intraocular pressure and this high pressure seems to be caused by a certain disturbance in the circulation in the eye. The fact that these patients are born with normal eyes and do not become blind until later in life shows that lack of light has no share in the development of this hereditary disease or mutation. The question then remains, how can we account under such an assumption for the fact that blind animals are so prevalent in caves and so rare in the open? We shall return to this question later on.

II.

We will now review briefly the literature dealing with observations and experiments on the influence of light on the formation of eyes. The fact that the eyes of mammals are formed in complete darkness (in the uterus) may serve at the outset as a warning against overestimating the effect of light on the formation of eyes. F. Payne[1] has raised sixty-nine generations of a fly

[1] F. Payne, BIOL. BULL., XXI., p. 297, 1911.

(*Drosophila*) in complete darkness without noticing any changes in their eyes or in their sensitiveness to light.

Recently Uhlenhuth[1] has demonstrated in a very striking way that the development of the eye does not depend upon the influence of the light or upon its functioning. He transplanted the eyes of young salamanders into different parts of their bodies where they were no longer connected with the optic nerves. The eyes after transplantation underwent a degeneration which was followed by a complete regeneration. Uhlen-

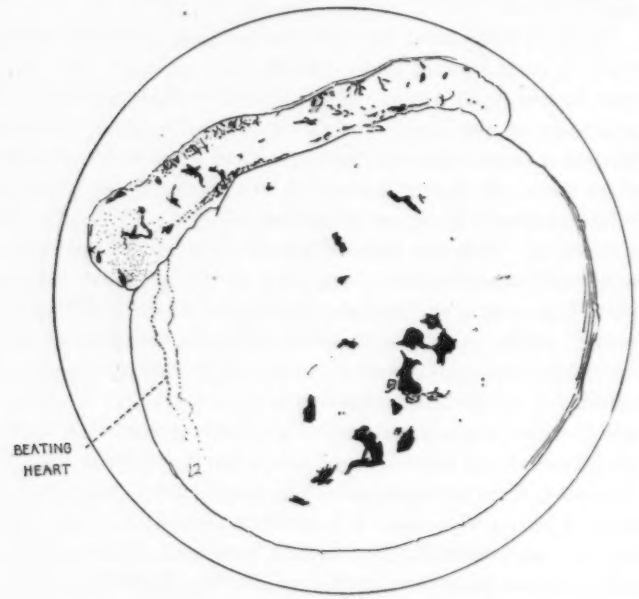


FIG. 1.

huth showed that this regeneration took place in complete darkness and that in salamanders, kept in the dark for 15 months, the transplanted eyes remained normal. Here the eyes were no longer in connection with the central nervous system, received no light, and could not possibly have functioned and yet they regenerated and kept normal. The degeneration which took place in the eyes immediately after they were transplanted was

[1] Read at the meeting of Anatomists at St. Louis, December 1914.

apparently due to the interruption of the circulation in the eye and the regeneration probably set in with the reëstablishment of the circulation in the transplanted organ.

The older literature had many observations which were assumed to prove an influence of light on development but the writer has shown in a former paper that these conclusions were not supported by the facts on which they rested.

The writer has for years paid special attention to the possibility that light might influence the formation of organs in

FIG. 2.

animals, but he has succeeded in finding only one form in which such an influence can with certainty be demonstrated, namely the hydroid *Eudendrium*. In his experiments in Naples he had noticed that the polyps of this form regenerate better in the light than in the dark and in Woods Hole he could convince himself that the stems of *Eudendrium* cannot regenerate their polyps if kept permanently in the dark.[2]

[2] Loeb, "Über den Einfluss des Lichtes auf die Organbildung bei Tieren," *Arch. f. d. ges. Physiol.*, LXIII., p. 273, 1896; Goldfarb, *Journal Experimental Zoology*, 3, 129, 1906; 8, 133, 1910.

Kammerer[1] seems to be the only author who still takes it for granted that cave animals owe the degeneration of their eyes to lack of light, and his support for this view consists in the statement that five young cave salamanders (*Proteus*) developed larger eyes under certain (somewhat puzzling) conditions of illumination. In other cases the eyes of *Proteus* remained unaltered in the light. It would be advisable to make certain whether or not there are two varieties of *Proteus* and moreover it would be desirable to repeat these experiments on a larger scale.

The writer wishes to publish in this note the results of some experiments made in 1912, which prove that in the fish *Fundulus* it is comparatively easy to produce embryos with degenerated eyes by various means except by the one Kammerer holds

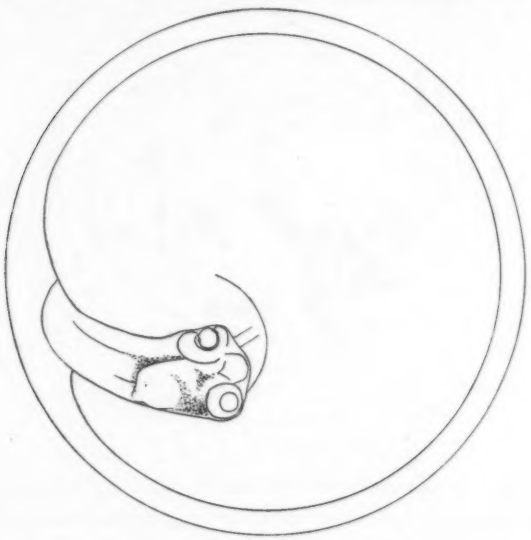


FIG. 3.

responsible for this phenomenon, namely lack of light. The reader may in passing be reminded of the fact that in this form Stockard and later McClendon induced the formation of cyclo-

[1] Kammerer, *Arch. f. Entwicklngsmechn. d. Organ.*, XXXIII., p. 350, 1912.

pean eyes by altering the constitution of the sea water (addition of magnesium salts or of alcohols and other narcotics.)[1] The writer's attention to an abnormal development of the eyes in this embryo was first attracted in his experiments on heterogeneous hybridization.[2] He noticed that among the anomalies noticeable in heterogeneous fish hybrids the lack of circulation was the most common, but that incomplete or abnormal development of the eyes was not infrequent. Such embryos often give the impression that they have no eyes at all, though in reality a histological analysis would probably show that some of the organs of the eye are present. But it is safe to say that the

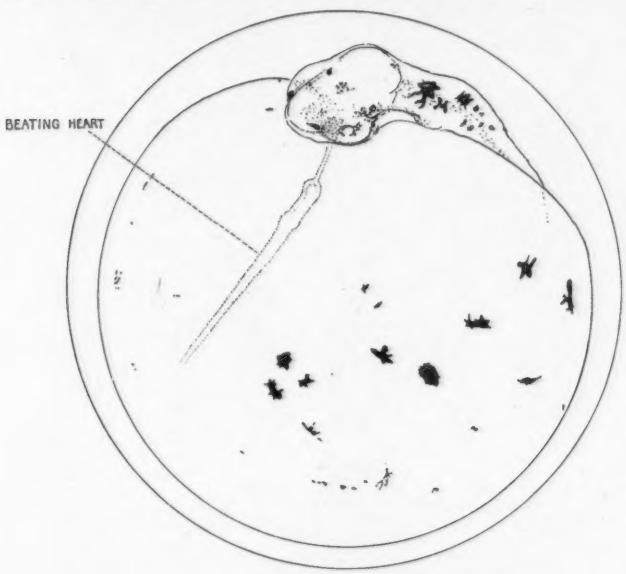


FIG. 4.

condition of the eyes is such as to make them unfit to form a retina image and for this reason we may call these embryos with abnormal (or apparently lacking) eyes blind; in the same sense in which this term is used in the case of the blind salamanders or

[1] Stockard, *Am. Jour. of Anatomy*, 10, 369, 1910; *Jour. Exp. Zool.*, 4, 165, 1907; 6, 286, 1909; McClendon, *Am. Journ. Physiol.*, 29, 289, 1912.

[2] Loeb, "Heredity in Heterogeneous Hybrids," *Jour. Morphol.*, XXIII., p. 1, 1912.

fish found in caves which also have some of the elements of an eye but in a condition unfit for use. The term "blind fish" is therefore used in the following as synonym with imperfect or degenerated eyes.

III.

Blindness Produced through Heterogeneous Crossings.

Figs. 1 and 2 are camera drawings of hybrids between *Fundulus heteroclitus* ♀ and *Menidia* ♂. The first embryo is eight, the second fourteen days old. In neither of these two embryos are eyes noticeable, though of course a histological examination might have divulged rudimentary eyes. The embryos are in this

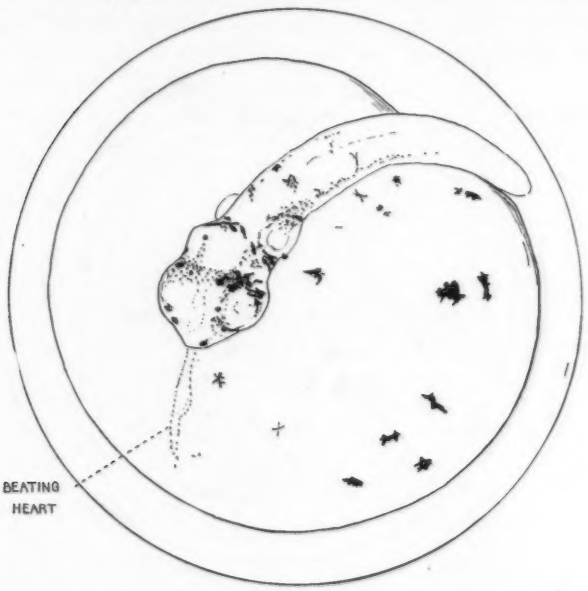


Fig. 5.

respect to all appearances comparable to the blind cave fish or salamanders.

It is a known fact that certain blind animals have in an early stage a comparatively better developed eye than in later stages, *i. e.*, that the eye either stops developing early while the development of the rest of the body continues or that the eyes degenerate

directly. The writer noticed not infrequently that the eyes of the cross between *Fundulus* and *Menidia* were normal in the beginning but appeared more abnormal the further the development progressed. We will illustrate this statement by two series of drawings. The first case is illustrated by Figs. 3 to 6. Fig. 3 shows the embryo four days old. The embryo has normally developed eyes with lenses. The embryo's heart, which is visible in front, was not yet beating and no pigment was formed. The same embryo is shown two days later in Fig. 4. The eyes instead of developing any further are less distinct. Fig. 5 is the same embryo two days later (eight days old). The eyes are

FIG. 6.

hardly recognizable, it is difficult to decide without sectioning whether the lens still exists. The internal ear is distinguishable. The heart is pulsating.

Fig. 6 shows the same embryo at the age of two weeks. Apparently the pigment is the only organ of the eye which is developed.

Figs. 7 and 8 illustrate the same fact, that a rather normal eye with a lens was formed while later on only a very imperfect eye remained. Fig. 7 shows the embryo when four days old with a lens and an apparently normal eye, while five days later (Fig. 8) no eye was perceptible.

IV.

Blind Fish Produced from Pure Breeds of Fundulus Heteroclitus.

The writer was under the impression that the abnormalities in the development of the heterogeneous hybrids in fish were due to the influence of the foreign sperm as in the similar case of the

Fig. 7.

fertilization of the egg of the sea urchin with the sperm of the star fish, where the mortality is also enormous. In order to prove this assumption he tried to find a method which would allow him to produce embryos with abnormal eyes in the pure breeds of *heteroclitus*. He has already mentioned in a former publication that such abnormal embryos were obtained when

their development was retarded by putting them into sea water to which some KCN was added. Fig. 9 shows a blind embryo obtained in this way. The eggs were put immediately after fertilization into a weaker solution of KCN in sea water where they remained sixteen days. They developed slowly. The embryo was drawn two days after it had been put back into

FIG. 8.

normal sea water. The heart-beat and circulation were established. This method of producing blind embryos is not reliable and need not be discussed any further.

A better method was found by exposing the newly fertilized eggs to a low temperature (from between 0 to 2° C.) for some time. The writer found that the egg of *Fundulus* can be put for weeks into such a low temperature after the embryo is once formed, without any injury to the latter. As soon as it is put back to room temperature it continues to develop. This corresponds with the idea that the low temperature only retards the chemical reactions underlying development. If, however, the

egg of the same fish is exposed to such a low temperature immediately after fertilization or when the eggs are in the process of early segmentation they suffer severely.

Thus in one experiment the eggs of *Fundulus* (fertilized with sperm of their own species) were distributed into a number of Erlenmeyer flasks and put on ice immediately after fertilization and kept at a temperature of between 0 and 2° C. After four

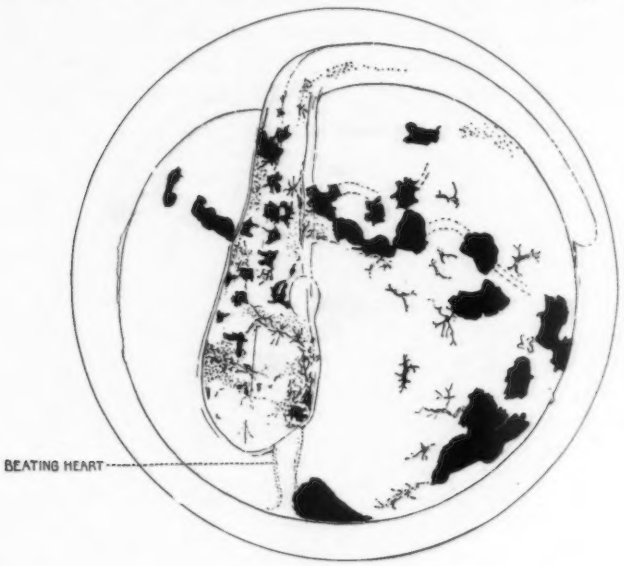


FIG. 9.

hours, seven and a half hours, twenty-three hours, thirty-two hours, forty-eight hours, sixty-four, and ninety-six hours one lot was returned to room temperature. All the eggs taken out after forty-eight hours or later were dead; few of those taken out after thirty-two and twenty-three hours survived. A considerable percentage were abnormal and resembled the heterogeneous hybrids between *heteroclitus* ♀ and *Menidia* ♂. Most of those taken out after four and seven and a half hours survived, but about 20 per cent. to 30 per cent. were abnormal. Among the abnormal embryos a number of blind fish were found.

A second lot of eggs were put on ice four and a half hours after fertilization when they were in the 4-cell stage or beyond. These eggs were just as sensitive to the effect of the low temperature as those put on ice immediately after fertilization.

This experiment was repeated four times with the same result. In another experiment the eggs were allowed to develop to about the 128-cell stage at normal temperature before they were put into a temperature of 0° (about fifteen hours after fertilization). Eggs that were kept at that low temperature for

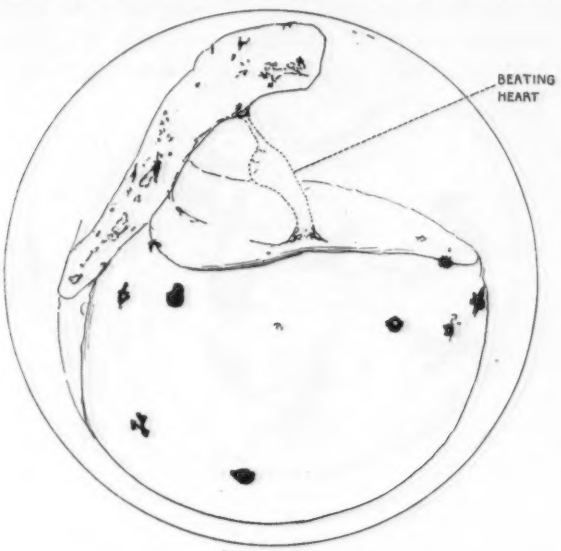


FIG. 10.

two days were still able to develop into normal embryos but those that had been kept three days at the temperature of from 0 to 2° C. were practically all dead.

When the eggs were put into a temperature of from 0 to 2° C., after the embryo was formed and the circulation established, they could resist the low temperature for weeks. When put back to normal temperature they recovered and developed normally.

Further experiments are required to ascertain more accurately when the eggs become immune to the temperature of from 0° to 2°.

It is only this very low temperature at or very near the freezing point which is injurious for the newly fertilized eggs. If the temperature is a little higher, *e. g.*, 7° C., the newly fertilized eggs can live for weeks in it without being injured. Thus in the experiment just mentioned some of the eggs were put immediately after fertilization into a temperature of about 7° C. These eggs

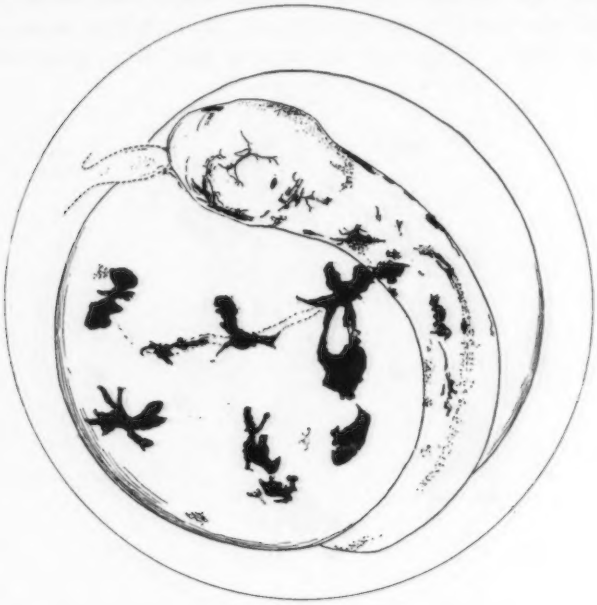


FIG. 11.

developed very slowly but no abnormal embryos were observed although some of the eggs were kept at a temperature of 7° C. for four weeks. Those that were kept still longer at that temperature suffered but probably not from the low temperature but from the fact that the flasks in which they were kept were closed.

These experiments thus establish the interesting fact that immediately after fertilization the eggs of *Fundulus* are rapidly injured or killed when exposed to a temperature of 0° or a little above, while after the embryo is formed they can be exposed to

such a temperature for a month without suffering. The newly fertilized eggs can, however, be exposed without injury to a temperature of 7° for weeks and possibly longer.

As already stated, among those eggs which were put into a temperature of from 0 to 2° C. a certain number had defective eyes. Thus the egg Fig. 10 was put immediately after fertilization into a temperature of between 0 and 2° for twenty-four hours and then put back to room temperature. The egg developed like a typical heterogeneous hybrid. Fig. 10 represents the embryo when two weeks old; it had a beating heart but no circulation. No eyes are noticeable. Fig. 11 represents an embryo from another experiment; in this too the egg had been

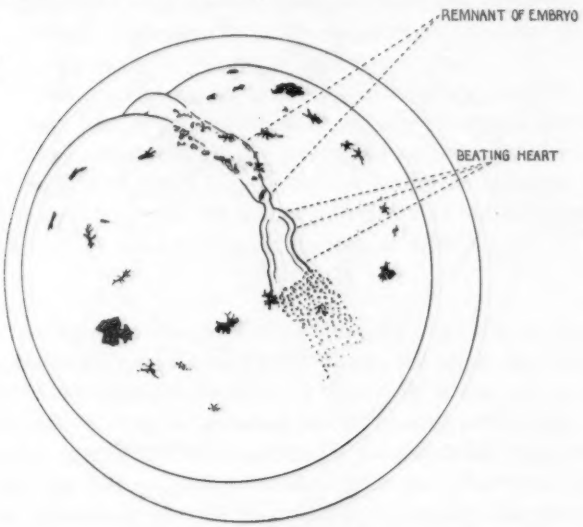


FIG. 12.

kept for four hours (immediately after fertilization) on ice (0 to 2°). Apparently no eyes are formed but the circulation is established.

Figs. 12 and 13 are added to show to what extent the abnormal embryos, produced by a short exposure of the egg to a very low temperature (0°) immediately after fertilization, resemble the monstrosities which are formed in the case of heterogeneous

hybridization. The embryo in Fig. 12 consisted practically only in a pulsating heart, what was left of the embryo was much less conspicuous than it is in the drawing. The embryo was ten days old. The egg had been put into the temperature of between 0 and 2° when it was in the 2-cell stage and had remained there for twenty-four hours.

The remnants of the embryo Fig. 13 had the same history. In

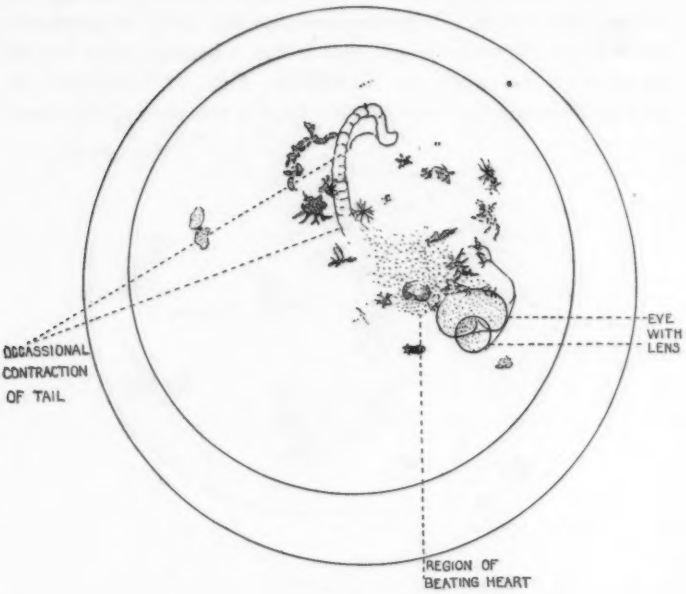


FIG. 13.

this case, curiously enough, one eye with a lens and the tail was all that could be recognized. The monstrosity when drawn was eleven days old. An inexperienced observer might easily have concluded that in this case only the eye had been developed, while in reality the whole embryo had developed but the eye had survived while other parts perished.

The analogy with teratomata is obvious.

V.

The writer was anxious to know whether it was possible to produce deficiencies in the eyes of *Fundulus* by raising them in the dark. It was necessary to carry on such an experiment with a large number of embryos. Since it was possible that a short exposure of the unfertilized egg or of the sperm to the light might already have some effect, the females and males of *Fundulus heteroclitus* to be used for the experiment were put into an absolutely dark room where everything was prepared for the experiment. The females and males were stripped of their sexual cells in the dark and the jars containing both sperm and eggs were put into dark boxes and kept in the dark room for four weeks. Although many embryos had died, hundreds had survived. All had perfectly normal eyes. This experiment confirms similar experiments made by the writer in previous years.

All these experiments show that while it is comparatively easy to produce blind *Fundulus* embryos, or, more correctly speaking, fish with degenerated eyes, by heterogeneous hybridization or by low temperature or by lack of oxygen (or by an excess of magnesium salts or by alcohol, as shown by Stockard and McClendon) no such result can be produced by lack of light.

VI.

If we consider all the facts in the case there is nothing at present to warrant the assumption that the blind cave animals owe the deficient development of their eyes to the lack of light, since lack of light is according to our present knowledge a less efficient agency in the causation of an abnormal development of the eye than a number of other injurious influences. Under these conditions we must be prepared to consider the possibility that many if not all the blind species found in caves owe their blindness to other influences than those of the cave. Eigenmann states that no blood vessels enter the eye of the blind cave salamander *Typhlotriton*. Since the experiments of Uhlenhuth as well as those of Stockard and of the writer reported in this paper indicate the importance of the circulation and of chemical factors in the development of the eyes it is not impossible that in the blind fish (Amblyopsidæ) as well as in the blind sala-

manders a hereditary disturbance in the circulation or nutrition of the eye or its surroundings is the cause of the degeneration of the eyes. There remains then the one difficulty mentioned in the beginning, namely to account for the fact that the relative number of blind species is greater in caves than in the open.

Eigenmann has shown that all those forms which live in caves were adapted to life in the dark before they entered the cave. These animals are all negatively heliotropic and positively stereotropic and with these tropisms they would be forced to enter the cave whenever they are put at the entrance to the cave. Even those among the Amblyopsidæ which live in the open have those tropisms of the cave dweller. This eliminates the idea that the cave adapted the animals for the life in the dark.

Eigenmann's observation makes it also clear that blind animals are comparatively rare in the open and that animals with normal eyes are not in the majority in the caves. Only those animals can thrive in the caves which for their feeding and mating do not depend upon visual mechanisms; and conversely animals which are not provided with visual mechanisms can only under exceptional conditions hold their own in the open where they meet the competition of animals which can see. This would account for the fact that in caves blind species are comparatively more prevalent than in the open.

In spite of all this, Eigenmann is inclined to assume that the darkness of the caves was a factor in promoting the blindness of the cave fauna. While those Amblyopsidæ which live in the open have already abnormal eyes those species of the family which are found in the caves have more degenerated eyes than those in the open and Eigenmann is inclined to ascribe this fact to an accumulated influence of the darkness. This would, however, compel us to account in a similar way for the incipient degeneracy of the eyes of those Amblyopsidæ that have never been found in caves and for the complete blindness of *Typhlogobius;* and would leave us at a loss to account for the presence of salamanders with perfectly normal eyes in the caves. It seems to the writer that consistency would demand to consider a common mode of origin of all these blind forms, namely as mutations, *i. e.*, as the result of some factorial change in the germ the cause and nature of which we are not yet able to define. Among the

Amblyopsidæ various mutations in the state of the eye may be constantly arising (as in the case of *Drosophila*); and some of these mutants, especially those that are perfectly blind, can not hold their own in the open while in the caves they can preserve and perpetuate themselves. This assumption would not exclude the possibility that Kammerer's observation may have been correctly interpreted by him, it would only provide for the possibility that his conclusion can not be generalized—a possibility which from the experiments mentioned in this paper must be seriously considered.

SUMMARY.

1. It is shown that blind embryos or more correctly embryos with degenerated eyes can be produced by heterogeneous hybridization in fish embryos (*e. g.*, *Fundulus heteroclitus* ♀ and *Menidia* ♂). Since in these cases as a rule no circulation exists the inference is possible that the anomalous condition of the eye may be due to lack of circulation.

2. Blind embryos of the pure breed of *Fundulus heteroclitus* may be produced by the addition of KCN to the sea water.

3. It is shown that immediately after fertilization (by sperm of their own species) and during the early stages of segmentation the egg of *Fundulus heteroclitus* is rapidly killed or injured if it is exposed to a constant temperature of about 0° (or slightly above); while it may be exposed to a slightly higher temperature (*e. g.*, 7° C.) for weeks without being injured. If the egg is exposed to the low temperature after the embryo is once formed it can resist the low temperature of from 0 to 2° C. for weeks without permanent injurious effects.

4. If eggs of *Fundulus heteroclitus* are fertilized with the sperm of the same species and exposed immediately after fertilization for a number of hours or a day to a temperature of between 0 and 2° C. abnormal embryos can be produced a certain percentage of which may show degenerated eyes.

5. Lack of light does not influence the development of the eyes of *Fundulus*.

6. It is pointed out that internal mutational changes and not lack of light may account for the blindness of certain cave fish and salamanders.

THE ABSORPTION OF FAT BY FRESHWATER MUSSELS.[1]

E. P. CHURCHILL, JR.

INTRODUCTION.

The present paper embodies the results of the first of a series of investigations designed to ascertain whether or not animals living in the water use food which is in solution in the surrounding medium in addition to "formed" food such as plankton, etc. If it is found that such food in solution is used, it is of importance to learn whether it is absorbed by the alimentary tract alone or to some extent by the epithelium of the outer body walls, especially that of the gills in the case of mussels.

Pütter ('08) was the first to advance the theory that food could be so taken. He based his conclusions in part on a comparison of the amount of carbon necessary for the maintenance of the organism with the amount furnished by the plankton. Pütter considered the latter too small for the needs of the animal and argued that it must use some carbon which is in solution in the water, resulting from the decay and disintegration of organic life. He further stated that the amount of material found in the alimentary canal is never large enough to supply the requisite quantity of carbon. Besides the alimentary canal, the uncutinized epithelium of the outer surface of the body, especially that of the gills, was thought to function in absorbing dissolved food. This process went on in addition of course to the digestion of "formed" food by the alimentary canal. Pütter concluded that the above conceptions applied to Protozoa, Porifera, Echinoderms, Crustaceans, Mollusks and Fishes. He tested the matter experimentally in two ways: first by noting that goldfish and perch lived much longer in solutions of asparagin, somatose and glycerin than in tap water: secondly by com-

[1] Contribution from the United States Biological Station, Fairport, Iowa. (Two of the experiments were carried out at the zoölogical laboratory of the Johns Hopkins University.) Published by permission of the Commissioner of Fisheries.

paring the amount of oxygen needed to oxidize the lost weight of tissues of actinians, tunicates and fish while kept in their natural medium with the amount of oxygen actually used. As the latter was found to be greater than the estimated quantity needed he concluded that the extra oxygen was used in oxidizing some food that had been taken from the water where it was present in the form of a solute.

Knorrich (10), working with *Daphnia*, which lived 14 days in sterilized hay solution, concluded that nutriment was absorbed from the solution.

Kerb (10) kept eels in sugar solution and noted no diminution of the amount of sugar from day to day. He obtained similar results while working with *Corethra* larvæ in sugar solutions. Also he found that *Daphnia* lost in dry body weight as rapidly in solutions of peptones as in tap water.

Wolff (10), working with *Simocephalus*, found that it lived twice as long in bacteria-free water, which contained some dissolved carbon compounds, as it did in tap water. He made no observations as to body weight lost or gained.

Lipschütz (13) reviewed the entire subject including his own previously published experiments along that line and offered criticism of Pütter's work. Lipschütz noted that fish and eels when kept in nutrient solutions lost as much weight as in tap water. He also thought that Pütter overestimated the amount of material in solution in the water and underestimated the carbon content of the plankton. His general conclusions are the opposite of those of Pütter.

Lund[1] found that if Protozoa are kept in a weak soap solution they will absorb fat from such solution through their body walls. At his suggestion the fats used in the following experiments were rendered soluble in water by saponification.

I am especially indebted to Dr. Caswell Grave, at whose suggestion the work was undertaken, for his advice and aid on many occasions and his supervision of the preparation of the manuscript. I also desire to express my obligations to Dr. E. J. Lund for suggestions concerning some of the chemical reactions

[1] Dr. Lund's paper is not yet published, his results having been communicated to me verbally.

discussed in the latter part of this paper; to Dr. G. L. Houser for the use of several books and a microscope from the laboratories of the University of Iowa; and to Dr. R. E. Coker for aid rendered while the work was being carried on at the Fairport Laboratory.

Materials and Methods.

The mussels upon which the investigations were carried out were individuals selected from the more common species found in the Mississippi River near Fairport, Iowa. Both adult and juvenile specimens were employed. Care was exercised to choose for the experiments non-gravid mussels which were in a seemingly healthy condition, "shoulder-raked" or hand collected individuals being generally used in preference to those dragged out of the water by "crow-foot" hooks.

The water used in the experiments was that of the general supply for the Fairport Laboratory which had come from the Mississippi River through a filter which removed the mud and at least the greater part of the living animal and plant organisms.

The fats employed were those of olive oil and cottonseed oil. The results were the same in both cases as far as could be observed. The oils were saponified by boiling with sodium hydroxide, the resulting solution being diluted to strengths varying from .001 per cent. to .005 per cent. in different cases. This method can be used only with freshwater animals as the soap is precipitated from the solution in salt water.

The adult mussels experimented with were kept in glass aquaria containing about 5,000 c.c. of the fat solution of the desired strength. The bottoms of the aquaria were covered to a depth of an inch or two with coarse sand. Control individuals were run side by side with the experiments in similar aquaria containing filtered water only. The juvenile mussels were generally kept in smaller aquaria. In the cases of experiments extending over more than twenty-four hours, the solutions were changed daily, no effort being made to balance the aquaria. As the mussels from the river are accustomed to a current of water this seemed to be the closest approximation to natural conditions which it was possible to obtain in the laboratory.

The tissues of various portions of the adult animals experi-

mented with and of the control mussels were sectioned by the freezing method, stained with Sudan III. and mounted in glycerin. Also sections of the tissues of the adult and of the entire bodies of the juvenile mussels were made after fixation in osmic acid and Muller's fixing fluid. In this method the higher alcohols were avoided by clearing the sections in clove oil from 85 per cent. or 90 per cent. alcohol and infiltrating in paraffin from the clove oil. The paraffin was removed with xylol and the sections were mounted in xylol balsam. Such sections were studied very shortly after mounting, as the fat is gradually dissolved out by the xylol.

In some cases the fat solution was stained by dissolving Sudan III. in the same to the point of saturation. The external appearance of various parts of mussels which had been kept in such stained solutions was noted. Also frozen sections of such mussels were examined to ascertain whether or not stained fat had been absorbed from the solution. In such cases the sections were mounted in glycerin directly after cutting.

Observations.

The mussels were found to live readily in .001 per cent. to .005 per cent. fat solutions. The only abnormal manifestation to be observed was that a considerable quantity of mucus was thrown off. Six adult individuals which were kept in fat solution for one month were living at the close of that period. At the expiration of the same length of time one of the control mussels of this experiment had died. After the adoption of the method of manipulating the aquaria outlined above, no mussels died while kept in fat solutions varying in strength from .001 per cent. to .005 per cent. However, beyond the fact that mussels can live for at least one month in fat solutions of these strengths, no evidence was secured concerning the relative longevity of those kept in fat solutions and of the control individuals. No weights were taken. Experiments calculated to ascertain whether or not the absorption of fat from the solutions employed is of advantage to mussels were deferred until the actual entrance of the fat into the tissues of the animal was proved. Emphasis was therefore laid upon the histological evidence of the absorption

of fat and it is such evidence that this paper is designed to set forth.

Mussels which had been kept in unstained fat solution will be considered first. Camera lucida drawings were made of typical portions of the sections, care being taken to indicate as far as possible the number and position of the fat droplets.[1] In some cases the fat was so massed together that individual droplets were indistinguishable. Fat is represented in all the drawings by heavy black dots or areas.

The case of an adult specimen of *Quadrula ebena* which had been kept in .001 per cent. fat solution for 15 days will first be discussed. Sections of the gill filaments of this mussel were prepared after fixation in a solution of osmic acid and Muller's fixative. In such sections an abundance of fat droplets was to be seen in the epithelial cells and a number in corpuscles in the blood vessel in the interior of each filament (Fig. 1, Pl. I.). Very few fat droplets were found in sections of the control individual prepared by the same method (Fig. 2, Pl. I.). Frozen sections, after having been stained with Sudan III., gave results parallel to those just stated—a large number of fat droplets in the gill filaments of the mussels which had been kept in fat solution for 15 days (Fig. 3, Pl. I.), but practically nothing that took the Sudan III. stain in the control individuals. The epithelial lining of the water tubes of the gills of the individuals which had been kept in fat solution was also quite crowded with fat. In sections of the intestine of mussels which had been kept in fat solution, fat droplets appeared in the epithelial lining. Also in sections of palps and mantle fat droplets were revealed but not in such abundance as was true in the case of the gills. Fat droplets were quite numerous in the cells of the side of the mantle next to the body but there were practically none in the cells of the side lining the shell. The tissues of three adult mussels which had been kept in fat solution for 15 days and of two control individuals which had remained in filtered water for the same period were examined by the two methods of preparing

[1] The term "droplets" will in this paper be applied to the spherules of fat found in the tissues of the mussels which were studied. These droplets usually were of diameters varying from 5 to 10 microns, though in frozen sections of the gills instances were found in which the diameter was as great as 20 microns.

sections. The former all presented abundant evidence of the presence of fat. The difference between those which had been kept in the solutions and the control mussels was as striking as that shown in Figs. 1 and 2.

The best results were obtained from the study of juvenile mussels as in these cases the entire animal could be sectioned serially and the distribution of the absorbed fat over the entire body observed. A specimen of *Anodonta imbecillis* which had been kept in .002 per cent. fat solution for 10 days and a control specimen of the same species which had been kept in filtered water for the same length of time were selected as typical cases for study.

Figs. 4 and 5, Pl. II., showing corresponding parts of the foot, give an idea of the abundance and distribution of fat in the mussel which had been kept in the fat solution and of the almost entire absence of it in the case of the control specimen. (In this case as well as in that of several of the other figures, no effort has been made to draw the outlines of the epithelial cells, the intention being merely to represent the relative amount and distribution of the fat.) It will be noted that the fat is very abundant in the epithelium. It is present in some quantity in the interior of the folds of the foot and also within the deeper parts of the foot, apparently adhering to muscle fibers. The fat droplets exhibit some tendency here, and more markedly elsewhere, to gather together in clumps or to form chains. Several blood corpuscles which contain fat may be seen.

Fig. 6, Pl. II., shows a portion of the foot of the same individual, extending from the base of a fold to the blood vessel in the center of the foot. An abundance of fat was observable in the epithelium and quite numerous droplets appeared clinging to muscle fibers or enclosed within corpuscles in the central part of the foot.

Fig. 7, Pl. II., representing a portion of a fold of the foot, was drawn under the 1/12 oil immersion lens, an effort being made to depict the exact number and position and also the relative size of the fat droplets, muscle fibers and cells.

Fig. 8, Pl. I., shows in (*a*) the cross section of a liver tubule of the mussel used as the control, the few coarse stipples represent-

ing the fat present; and in (*b*) the cross section of the corresponding part of the mussel which had been kept in the fat solution. In the latter the liver cells are seen to be heavily loaded with fat, in some cases practically filled with it. A very great abundance of fat was found throughout the liver cells in all sections of this mussel.

Figs. 9, 10, and 11, Pl. I., are drawings made from the sections of the epithelial cells, respectively, of the intestine, of a gill filament and of the side of the mantle next to the body, of the specimen of *A. imbecillis* which had been kept in the fat solution for 10 days. They represent as accurately as could be drawn with the camera lucida the relative amount and arrangement of the fat droplets. Many corpuscles containing fat were found in the blood lacunæ immediately outside of the cells of the intestine. Some of these corpuscles were lying at the bases of the cells as shown in Fig. 9. Corpuscles containing fat were also found in nearly all blood vessels of the gill filaments. Many such corpuscles were in the position shown in Fig. 10, that is, close against the bases of the cells of the filament. In the mantle fewer corpuscles were seen. Here the fat was found adhering to muscle fibers and strands of connective tissue.

Figs. 12 and 13, Pl. III., represent mesenchyme cells and blood corpuscles of the mussel which had been kept in the fat solution and of the control individual respectively. In these sections may be observed the relation of the fat droplets to the tissues of the areas occupied by mesenchyme cells, which are especially numerous in the dorsal part of the body of the mussel. Fat was found scattered quite thickly throughout such regions in the case of the animal which had been in the solution.

The gill of an adult specimen of *Quadrula ebena* was sectioned immediately after it had been taken from the river. Fig. 14, Pl. I., represents the relative amount of fat found in its gill filaments. Somewhat more fat appeared in these sections than in those prepared from the tissues of the control mussels which had been kept in filtered water for 10 or 15 days but a very much smaller quantity was found than in the mussels which had been kept in fat solution.

The mussels which had been kept in the fat solution that had

been stained with Sudan III. will now be considered. Before beginning the experiment the valves of the shell were opened sufficiently to permit the observation to be made that the tissues of the animal to be experimented with were of normal color and condition. The mussels were then kept in stained fat solution for periods varying from 5 to 15 days. At the expiration of these periods the gills of the adult and the gills, mantles and foot of the juvenile animals were found to be red or pink in color. Sections of the gills of such adult mussels were prepared by the freezing method. In these sections pink colored fat droplets were found within the epithelial cells of the gill filaments and of the water tubes (Fig. 15, Pl. I.). This fact furnishes additional proof of the absorption of fat from the solution. It renders negligible the consideration that the heavy loading of fat found in each case in the mussels which had been kept in the fat solutions might have been due to the chance use of an extraordinarily fat individual. Some of the juvenile mussels which had taken on a red color while they had been kept in the stained fat solution were then transferred to filtered water. The color remained visible in the foot and gills for more than a week in some cases. This would tend to show that the red colored fat was absorbed and oxidized within the organism and that the red color was not due merely to the adherence of the stained solution to the surface of the gill or foot.

In all, including those mussels which were kept in stained and unstained fat solutions and the control individuals, the tissues of twelve mussels were examined. The sections all revealed much fat within the tissues of the mussels which had been kept in the fat solutions and very little or none in those of the control individuals. Consideration of the above results leaves no doubt of the fact that fat may pass from a fat solution into the body of the mussel. Now there remains the question of whether it is all absorbed through the intestine or partly through the tissues of the outer body walls. Experiments intended to throw light upon this question were undertaken with juvenile mussels. These had been kept in water containing little food for several weeks so that their tissues contained little stored fat at the beginning of the experiment.

Four mussels were kept in a .005 per cent. fat solution for the several periods of 4, 7, 18 and 24 hours. A small amount of fat was found in the epithelium of the intestine, mantle, gills and foot of the individual of the 4-hour period. None was found in the other parts of the body. About the same amount and distribution of fat was observable in the case of the mussel which had been in the solution for 7 hours. In the animal which was kept in the fat solution for 18 hours a very appreciable quantity of fat was found in regions corresponding to those in which it had been found in the mussels which had remained in the solution for 4 and 7 hours. A small amount was noted in the tissues immediately beneath the epithelium. None was found in the deeper body tissues, a fact which is in striking contrast to the case of the mussel which had been kept in fat solution for 10 days. The mussel which had remained in fat solution for 24 hours contained a considerable amount of fat in the same parts of the body in which it was found in the cases of the individuals which had remained in the solution for 4, 7, and 18 hours. Even more fat was found in the epithelium of the gills, mantle and foot than in that of the intestine (Figs. 16, 17, 18, 19 and 20, Pl. III.). A very small quantity of fat was observable in the liver cells. In certain parts of the foot a moderate amount of fat appeared immediately beneath the epithelium, some of which fat was adhering to the muscle fibers. The fat droplets became progressively less numerous as the tissues were studied to a greater depth within the foot and none were found among the muscle fibers or in the other tissues making up the main central mass of the foot (Fig. 17, Pl. III.). The corpuscles, especially those found in the gills, contained fat in many cases. In the gills the crowding of the corpuscles against the bases of the cells of the filaments was marked (Figs. 18 and 19). Every appearance was given that the fat was absorbed from the solution by the epithelial cells of the gills and was taken up from them by the corpuscles in some cases and in others thrown directly into the plasma of the blood. Fig. 20, Pl. III., shows a portion of the epithelium of the mantle. Fat was seen in the epithelial cells of the side next to the body and for a short distance beneath them but it was not found scattered throughout the deeper parts

of the mantle. No fat was found in the cells of the side of the mantle next to the shell.

The observations made on the mussels which were kept in fat solution for the periods of 4, 7, 18 and 24 hours may be summarized as follows:

1. More fat was found in the epithelium of the mantle, gills and foot than in that of the intestine.

2. In the case of the mussels which were kept in fat solution for 4 and 7 hours, fat was found only in the various epithelia.

3. In the mussel which remained in fat solution for 18 hours, much more fat than in the two above cases was found in the epithelium and a small amount was observable at various points beneath the epithelium, but none could be discerned distributed throughout the deeper body tissues.

4. In the mussel which had remained in fat solution for 24 hours, a very marked quantity of fat was found in the epithelium and a considerable amount appeared beneath the epithelium, diminishing in quantity, however, toward the interior of the body so that there was none distributed throughout the deeper body tissues.

5. No fat could be seen in the epithelium of the side of the mantle next to the shell but an abundance was present on the side which had been in contact with the solution.

6. Scarcely any fat was found in the cells of the liver.

From these facts it is probable that fat is absorbed by the outer epithelium of the body as well as by that of the intestine. Unless such were the case it would be very difficult to account for the presence of as much fat in the epithelium of the gills, mantle and foot as in that of the intestine, taking into consideration the fact that the mussels had been kept in the fat solution for such short periods, viz., intervals varying from 4 to 24 hours. It would also be difficult to account for the fact that such a very small amount of fat appeared within the deeper body tissues of these mussels while a very marked quantity was found in the epithelium of the outer body walls. It is very unlikely that the blood carried such a large amount of fat from the intestine to the outer epithelium in such a short time and during the same time carried none to the muscle fibers, mesenchyme cells or

connective tissue or conveyed none to the liver, which is the normal fat storing organ. If all the fat were absorbed by the intestine it seems certain that the sections of one at least of the mussels which had been kept in the fat solution for the short periods would have revealed more fat within the deeper body tissues than was found there. Wherever fat was found beneath the outer epithelium of these mussels which had been in the solutions for the short periods, much the larger quantity lay in close proximity to that epithelium and the amount lessened to zero toward the interior of the body. While the quantity of fat beneath the epithelium increased with the length of the time the mussel was kept in the solution, in all cases it was greatest nearer the epithelium. Apparently the fat found lying closely beneath the outer epithelium of the body had been absorbed and passed into the deeper tissues by that epithelium and had not come from the intestine. Also, if all the fat were absorbed by the intestine and the blood did transport it almost entirely to the outer epithelium it would seem most likely that the cells of the side of the mantle next to the shell would receive at least an observable amount.

In order further to test the question of whether or not fat may be absorbed from a fat solution by the epithelial cells of the outer body walls of the mussel the following experiment was performed. The valves of three adult mussels were wedged open with bits of wood and the animals suspended on a wire rack over the fat solution so that only the ventral parts of the mantle and foot were immersed. The mouth and siphons were above the solution so that it was not likely that any of the solution could enter the intestine by way of the oral or anal openings. One mussel was thus treated for 6 hours, the other two for 22 hours. They were compared with a fourth individual which had not been in the solution, all four mussels having been removed from the river only four or five days previous to the experiment. One of the animals which had been treated as above for 22 hours did not have much fat throughout the deeper body tissues but a moderate amount appeared in the epithelium of the part of the mantle which had been immersed in the solution. The other mussel which had been treated for 22 hours contained some

fat throughout the body and a heavy amount in the epithelium of the part of the mantle which had been exposed to the solution (Fig. 21*a*, Pl. III.). The control individual for this experiment contained much more fat throughout the deeper body tissues than the other three individuals but very little in the epithelium of the ventral part of the mantle (Fig. 21*b*, Pl. III.). The ventral part of the foot of the mussel which had been so treated for 6 hours revealed a moderate amount of fat in the epithelium but scarcely any in other parts of the foot. The fat found throughout the deeper body tissues of these individuals was of course previously stored fat, the mussels, as stated above, having been removed from the river but a short time. The point intended to be made is that a heavier loading of fat is found in the epithelium which had been in contact with the fat solution than in that which had not. While there is a possibility that some solution may have been carried by ciliary action up the mantle to the mouth, this probably did not occur as, in sections of the intestine, absolutely no fat was found in the epithelial cells. It seems highly probable that the epithelium of the mantle and foot absorbed the fat directly from the solution.

In regard to the mechanism of absorption of the fat by either the intestine or body walls little can be offered. The matter is quite complicated, some of the same possibilities entering here that serve to render the method of absorption of fat by the mammalian intestine as yet obscure. In some of the sections, especially in those cut by the freezing method, numerous droplets which took the stains used were found closely attached to the outer ends of the epithelial cells of the gills or mantle. These droplets were probably the fatty acids, a small part of which were present in the soap solution due to hydrolysis, by which process sodium hydroxide and the fatty acids would be formed; the remaining droplets were no doubt due to a slight acidity of the surface of the living cells resulting from the union of carbon dioxide from the cells with the water, forming carbonic acid. The fat may have been taken into the cells from these droplets upon the surface by phagocytic action or by solution in the plasma membrane, or it may have come directly from the sodium soap, the collection of the droplets upon the surface of the cells

being a phenomenon extraneous to the process of absorption. The latter interpretation seems the more probable as the droplets were not found upon the surface of the cells in nearly all the sections of tissues which had absorbed fat. The sodium may enter the cells with the acid radical and later be separated, or the fatty acid may be split off outside and enter the cell alone. As the fatty acid radical is the constituent of the fat molecule which takes the stain, only that radical could be followed in my preparations. In nearly all cases a fairly definite, though narrow, area lies between the outer ends of the cells and the fat clusters within. This band is the plasma membrane. The fact that no fat droplets could be observed actually in this membrane points to the conclusion that absorption of fat is effected by its solution in the plasma membrane and precipitation within the cell.

The manner of transportation of the fat within the body deserves mention. The bases of the cells of the intestine are in contact with blood lacunæ. Blood vessels traverse the gill filaments. A meshwork of blood lacunæ lies in the connective tissue beneath the epithelium of the mantle and foot. A considerable amount of fat is taken up and transported by the corpuscles to various parts of the body. Evidence of this is clearest in sections of the intestine and gills (Figs. 1, 9, 10, 18 and 19), in which corpuscles containing fat are found in close contact with the bases of the epithelial cells. Fat-loaded corpuscles are found in the blood spaces throughout the body of the mussel which had been kept in fat solution for several days. However, sections of the foot and mantle reveal many fat droplets lying immediately beneath the epithelium and clinging to muscle fibers or to the connective tissue instead of being contained in corpuscles (Figs. 4, 7, 11 and 17). No doubt, besides being transported by corpuscles, fat is thrown directly into the blood stream and carried thus to various parts of the body. In some cases the fat droplets may have become attached to muscle fibers, etc., as was observed in the sections, during the preparation of the tissues. In other cases the attachment may have occurred before the mussel was killed, the tissues to which the fat droplets were adhering being those which were later to oxidize the fat.

SUMMARY.

1. Fat which is in solution in water can be absorbed by fresh-water mussels.

2. Such absorption is accomplished by the epithelium of the intestine and also most probably by that of the gills, mantle and foot.

3. Fat is transported both by the blood corpuscles and by the plasma directly.

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EXPLANATIONS OF PLATES.

All drawings were made with camera lucida; Leitz Oc. No. 4, Obj. No. 6, unless otherwise indicated. The drawings are of sections prepared by the osmic acid method unless otherwise stated. Fat is represented by heavy black dots or areas.

PLATE I.

FIG. 1. Gill filament of adult *Quadrula ebena* after remaining in fat solution 15 days. *a*, epithelial cells. *b*, blood vessel. *c*, blood corpuscles. *d*, chitinous rods.

FIG. 2. Gill filament of the control for above figure: mussel had remained in filtered water 15 days. *a*, *b*, *c*, *d*, same as in Fig. 1.

FIG. 3. Gill filament of adult *Quadrula ebena* after remaining in fat solution 15 days. Frozen section, Sudan III.

FIG. 8. *a*, Cross section of liver tubule of juvenile *A. imbecillis* after remaining in filtered water 10 days, the control. *b*, Cross section of liver tubule of juvenile *A. imbecillis* after remaining in fat solution 10 days.

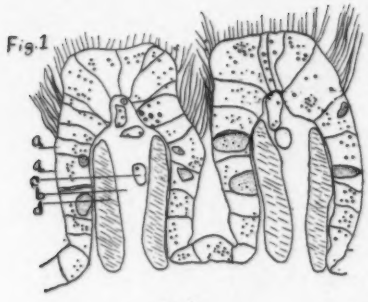
FIG. 9. Epithelial cells of anterior part of intestine of juv. *A. imbecillis* after remaining in fat solution 10 days. *a*, epithelial cells. *b*, blood corpuscles.

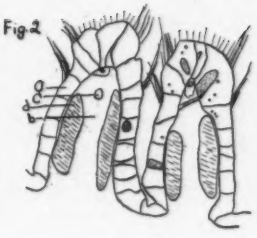
FIG. 10. Gill filament of juv. *A. imbecillis* after remaining in fat solution 10 days. *a*, blood corpuscles.

FIG. 11. Portion of epithelium on side of mantle next to the body of juv. *A. imbecillis* after remaining in fat solution 10 days.

FIG. 14. Gill filaments of adult *Quadrula ebena* sectioned immediately after taken from the river.

FIG. 15. Portion of gill filament of adult *Quadrula ebena* after remaining in Sudan stained fat solution 15 days. Black areas represent droplets of pink colored fat. Frozen section.





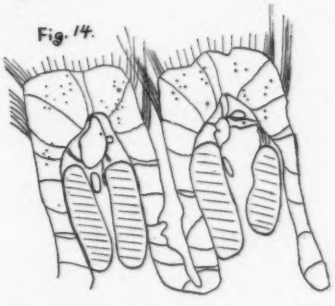


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PLATE II.

FIG. 4. Portion of foot of juv. *A. imbecillis* after remaining in fat solution 10 days. Epithelial cells not indicated. Fat shown heavily massed in epithelium and adhering to muscle fibers and in corpuscles. *a*, muscle fibers in cross section. *b*, blood corpuscles. *c*, muscle fibers in longitudinal section.

FIG. 5. Portion of juv. *A. imbecillis* after remaining in filtered water 10 days. Control for Fig. 4.

FIG. 6. Portion of foot of same individual as in Fig. 4. *a*, epithelium. *b*, muscle fibers in longitudinal section. *c*, muscle fibers in cross section. *d*, blood corpuscles.

FIG. 7. Portion of fold of foot of same individual as in Fig. 4. 1/12 oil immersion. *a*, epithelial cells. *b*, muscle fibers.

Fig. 7.

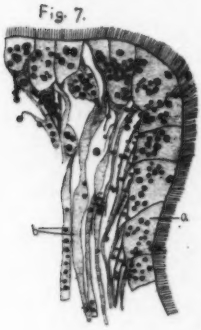

Fig. 4.

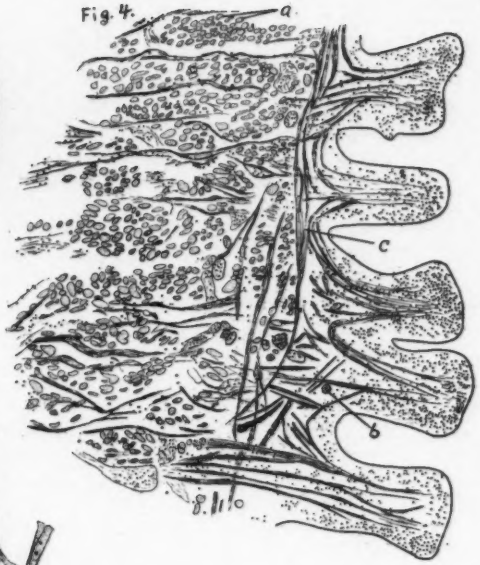

Fig. 6.

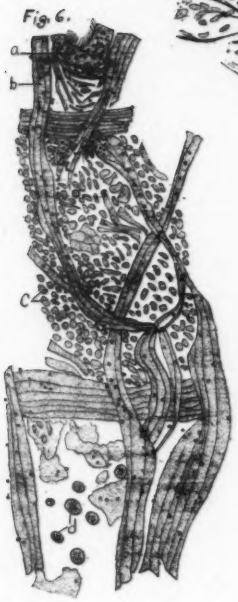

Fig. 5

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PLATE III.

FIG. 12. *a*, mesenchyme cells and *b*, blood corpuscles of juv. *A. imbecillis* after remaining in fat solution 10 days.

FIG. 13 *a* and *b*. Mesenchyme cells and corpuscles of the control kept in filtered water.

FIG. 16. Epithelial cells of intestine of juv. *Q. pustulosa* after remaining in fat solution 24 hours.

FIG. 17. Portion of foot of same individual as in Fig. 16.

FIGS. 18 AND 19. Gill filaments of same individual as in Fig. 16.

FIG. 20. Portion of side of mantle next to body of same individual as in Fig. 16.

FIG. 21. *a*. Epithelial cells of that portion of mantle immersed in fat solution 22 hours. The mouth of this mussel was kept above the solution. *b*. Epithelial cells of corresponding part of mantle of mussel used as control for Fig. 21 *a*.

Fig. 16.

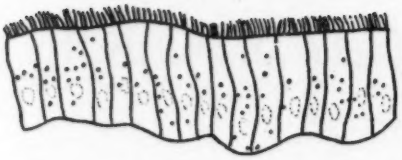

Fig. 17.

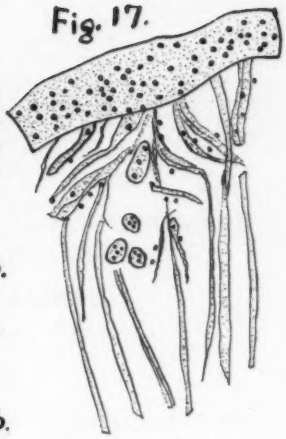

Fig. 12 a.

Fig. 12 b.

Fig. 13 b.

Fig. 21 a.

Fig. 13 a.

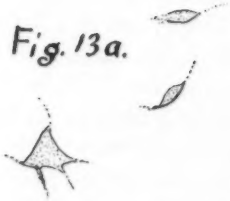

Fig. 18.

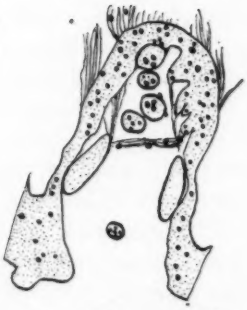

Fig. 21 b.

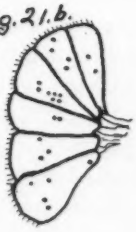

Fig. 19.

Fig. 20